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PATHOGENICITY OF BACILLUS MESENTERICUS, B. AROIDEAE, B. CAROTOVORUS, AND B. PHYTOPHTHORUS TO POTATO TUBERS

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A wound rot of stored potatoes was brought to the writer's attention by R. C. Wright in March, 1924. Some tubers that had been indexed for virus diseases and left in the laboratory for a day or two to cork over before being returned to cold storage developed a soft rot at the wounds made in indexing. The rotted tissue was white to cream colored, or brown at the margin. The texture was soft but elastic, not watery. No odor was detected. Two types of bacteria transferred from dilution were tested for pathogenicity to tubers. One of these proved pathogenic, was reisolated, and the reisolated organism again produced an active rot. Miss L. C. Cash,[1] of the Bureau of Plant Industry, U. S. Department of Agriculture, has referred this isolation to *Bacillus mesentericus* (Flügge) Migula.

Preliminary tests of pathogenicity were made by inoculating pure cultures of *Bacillus mesentericus* into needle wounds in sound tubers supported over water in loosely covered crocks at room temperature (about 20° C.). A black jelly-like rot developed in the form of a funnel about the needle prick. Sometimes this dried out after little progress, but at others it destroyed the entire tuber in from a week to ten days. The decayed tissue was usually covered with a brown, wrinkled surface growth and gave off a characteristic odor suggestive of mice. A culture of the blackleg *Bacillus* tested in parallel produced comparable amounts of rot at room temperature. As opportunity permitted, the comparison was extended to include two additional species of bacteria, various temperatures, and several varieties of potatoes. The work, still incomplete in many respects, has now been discontinued.

Parasitism of spore-bearing bacteria for potato tubers has been claimed by several workers but the evidence has not been generally accepted.

[1] The writer is indebted to Miss Cash for her determination of this organism, and also to Dr. J. I. Lauritzen for the use of inoculation chambers and for the records of temperature and humidity in these chambers during the experiments herein reported.

Kramer (8) early described a spore-bearing rod as the cause of a wet rot of tubers in Germany, but his evidence for pathogenicity of the organism rests on its capacity to decompose tubers immersed in potato extract cultures held at 35° C. for from 8 to 20 days. *Bacillus mesentericus vulgatus* is one of the bacteria reported by Lepoutre (10) to attack potato tubers. Van Hall (3) found *Bacillus subtilis* and *B. vulgatus* parasitic to potato tubers and to certain vegetables and nuts at high temperatures. He made isolations from the rot that developed when samples of garden soils were incubated on slices of potato, at 23°, 30°, 37°, and 42° C. *B. subtilis, B. vulgatus,* or both, were always recovered from the potato slices that rotted at 37° and 42°; no infection resulted at 23° or 30° C. When potato slices were inoculated with pure cultures of these organisms, both produced marked decay at 30° and 37° and *B. subtilis* invaded weakly at 23° C. The greater virulence and wider temperature range exhibited by the pure cultures was attributed to mass action.

Smith (19) has criticized this evidence for pathogenicity of spore-bearers on the grounds that the infection tests were made under conditions abnormal for the host and that none of the investigators showed pathogenicity of a strain derived from a single well-identified spore. This objection was met at least in part by the following simple test. Three freshly made subcultures in beef broth of the strain of *Bacillus mesentericus* under study, (a) unheated, (b) heated to 80° C. for 15 minutes, and (c) heated to 100° C. for 15 minutes, were incubated at room temperature. After 48 hours subcultures (a) and (b) showed the clouding and pellicle typical of the strain; (c) remained sterile, indicating considerably less heat resistance in this pathogenic strain than has been shown for some other strains of *B. mesentericus* (11). Dilution plates were made from (a) and (b), a single well-separated colony of each was fished, and the resulting cultures used to inoculate potatoes. Three tubers of the variety Earliest of All and two of Triumph were inoculated with each of these subcultures and a like number held as controls. After seven days' incubation at 30° C. typical rot 20–40 mm. in diameter developed in each of the inoculated tubers, and the controls remained sound (Fig. 1). No change in virulence was detected in the subculture heated to 80° C., which shows that the parasitic strain of *B. mesentericus* concerned here is considerably more resistant to heat than other known bacterial-tuber-rot organisms.

A single strain of *B. mesentericus* was used in this test and all other studies reported in this paper. This strain has never been single-spored but its purity has been repeatedly confirmed by the poured-plate method. No change in virulence has been detected during four years of cultivation on artificial media. A second isolation made from Virginia Irish Cobbler

potatoes by Freeman Weiss has proved equally virulent to tubers. No survey has been attempted, and it is not known whether pathogenicity to potatoes is a characteristic of *Bacillus mesentericus* as a species or of specialized strains only. The species has been recorded as present in mixed infections of blackleg rot and of bacterial ring disease by Paine and Haenseler (16) and Spieckermann (22) respectively, but neither tested it for pathogenicity. *B. mesentericus* is so common on imperfectly sterilized potato tissue that it is sometimes called the potato bacillus in American and European texts on bacteriology, but no previous mention of its pathogenicity has been found.

The three species of bacteria compared with *Bacillus mesentericus* as a measure of its pathogenicity under various conditions were *B. phytophthorus*, *B. carotovorus*, and *B. aroideae*. The identity of the culture here referred to as *B. phytophthorus* Appel (*B. atrosepticus* van Hall) which was isolated by the writer from a blackleg infected potato plant in a greenhouse at Arlington Farm, Va., has not been fully confirmed, but it is assumed to be the blackleg bacillus from its source and behavior. A subculture of

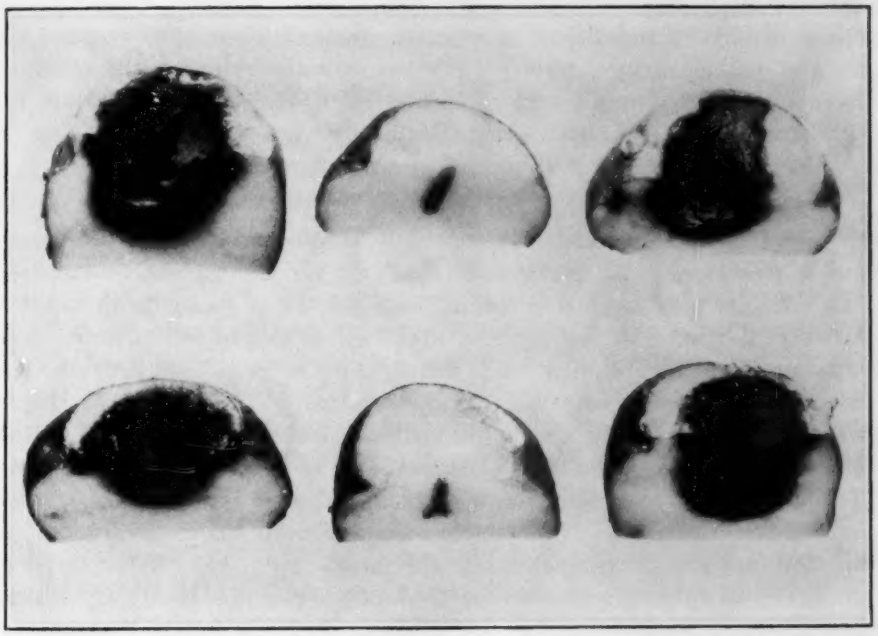

FIG. 1. Typical aspect of potato tuber rot caused by *Bacillus mesentericus*. Upper row: Triumph variety; lower row: Earliest of All. Left: Inoculated with unheated culture; center: control tubers pricked with a sterile needle (the darkening is due to wound cork formation); right: inoculated with a culture previously heated to 80° C. for 15 minutes. Incubated at 30° C.

Jones's original strain 3A of *B. carotovorus* Jones was obtained from J. I. Lauritzen, and a culture of *B. aroideae* Townsend from E. F. Smith's laboratory.

Bacillus carotovorus is now commonly named (18) as the cause of "slimy soft rot" of potato tubers, a loose term applied to bacterial soft rots, aside from blackleg, which commonly occur under conditions of handling and storage unfavorable to the tubers. Jones (6) described this organism as non-pathogenic to tubers under rigid test conditions, but other workers (9, 12, 21) have found it pathogenic in laboratory tests, and recently Lacey (9) has identified it as the cause of a soft rot of tubers under commercial conditions.

Bacillus aroideae has not been reported from soft-rotted potatoes under natural conditions, but several authors (12, 21, 23) report it pathogenic to tubers upon inoculation. This species has been reduced to synonymy with *B. carotovorus* (*Erwinea carotovora* (Jones) S. A. B.) by the Society of American Bacteriologists' Committee (1) on the basis of the findings of Harding and Morse (4). These findings show several minor differences in cultural characteristics between the two organisms which Harding and Morse considered insufficient to separate species. These authors made no tests of pathogenicity. Massey (12) has recently reopened the question by pointing out that *B. carotovorus* and *B. aroideae* differ in several respects, notably in their effect on the calla lily.[2]

TEMPERATURE RELATIONS ON CULTURE MEDIA

The four species, *Bacillus aroideae, B. carotovorus, B. phytophthorus,* and *B. mesentericus,* all grow well on beef agar and in beef broth. Tubes of broth or agar were inoculated with a 2-mm. loop from young broth cultures and placed at once at test temperatures. From two to five tubes of each organism were incubated at each temperature in each test, and all tests were repeated one or more times. The cardinal points for growth, which were alike for the solid and liquid media, are shown in table 1. At the lower temperatures negative records indicate no growth in three months. Temperatures given are the average of recorded observations to the nearest half degree Centigrade. The fluctuation was never more than 1° C. in any tests used in the compilation of the table.

Previous workers with *Bacillus phytophthorus* (13, 17, 20) agree that the minimum temperature for this organism is below 5° C. Smith (20) reports little growth below 4–5° C. The writer found a trace of growth on agar and distinct clouding of broth in three months at 2° C. The

[2] Link and Taliaferro (Bot. Gaz. 85: 198. 1928) report that "Serologically *B. aroideae* and *B. carotovorus* are distinct, although closely related."

TABLE 1.—*Temperature relations of tuber-rot bacteria in beef bouillon and on beef agar*

Organism	Minimum °C.		Optimum °C.	Maximum °C.	
	Negative	Positive		Positive	Negative
B. phytophthorus	0	2	28–32	34.5	37
B. carotovorus	0	2	28–34	37	39
B. aroideae	2	5.5	28–37	39	42
B. mesentericus	5.5	8	39–42	49	52.5

optimum as measured by rate of clouding broth was 28–32° in these tests; 25° or 28–30° according to others (1, 13, 17, 20). The maximum reported by Morse (13) is between 32° and 33°, by Shapovalov and Edson (17) between 33° and 35°, by Smith (20) 36° C. For *B. carotovorus* Jones (6) gives maximum 38°, optimum 27–30°, and minimum near 4°, negative at 1° C. For *B. aroideae* Townsend (23) gives minimum 6°, optimum 35°, maximum 41° C. The writer's records are in general accord with published reports in all cases. The data are presented here in tabular form to show the ascending sequence of cardinal temperatures from *B. phytophthorus* to *B. mesentericus*, a sequence that also holds for the growth of these organisms on potato tubers.

EFFECT OF TEMPERATURE ON DEVELOPMENT OF TUBER ROTS

Temperature relations of the bacteria on raw potato were determined by inoculating whole tubers and incubating them in special chambers in which temperature and humidity were under control.[3] A lot of five tubers in a separate 4-quart till basket was used for each organism and for a control at each temperature. A slice was cut from one end of each tuber with a sterile knife and this freshly cut surface was pricked with a heavy platinum needle bearing surface growth from a young agar culture. Control tubers were pricked in like manner with a sterile needle. Temperatures are presented as the average to the nearest half degree Centigrade and relative humidities as the average to the nearest per cent. Fluctuation during an experiment rarely exceeded 1° C. in temperature or 5 per cent in relative humidity.

A preliminary test of the four pathogens on the Green Mountain variety through a range of 8° to 34° C. showed *Bacillus aroideae* and *B. mesentericus* to be virulent at temperatures above 23° C., *B. phytophthorus* active at 8°–34° but especially at 8°–25°, and *B. carotovorus* weakly parasitic throughout.

[3] Wooden chambers about one meter in each dimension, with electrical heat control, air circulation, and manual regulation of humidity, with a permanent wet and dry bulb device for reading humidity in place, were available. Records of temperature and humidity were made twice daily.

This experiment was repeated with tubers of the White Rural variety. A slice was cut from the bud end and inoculation made by pricking a 3-day agar culture into the tuber to a depth of 3–3.5 cm. at right angles to the cut surfaces. The incubation period was 10 days. As a criterion of amount of rot two diameters were measured, one at the surface, the other parallel to the surface at a depth of 1 cm. Both measures are desirable in presenting an index of the amount of actual rot because of the different character of invasion by the several organisms. *Bacillus carotovorus, B. mesentericus,* and under some conditions *B. aroideae* may form craters or funnel-shaped cavities which decrease in diameter below the surface. On the other hand, the rot caused by *B. phytophthorus* frequently increases in diameter as the depth increases.

Plates XIX and XX show the character of rot resulting in this test after three days at eight different temperatures. Lateral extension of the cavity below the surface is shown in B. phytophthorus rot at 25° C. *B. aroideae* and *B. carotovorus* formed craters or funnels of rot at the lower temperatures, and *B. mesentericus* produced this type of cavity throughout the range. The photographs fail to differentiate clearly between cork formation or dark stains and actual rot. *B. phytophthorus* and *B. carotovorus* produced definite rot at all temperatures, *B. aroideae* a bare trace at 8° and definite invasion at higher temperatures, but *B. mesentericus* is definitely negative at 8° and made only a trace of development at 12° and 15.5°, the first definite rot appearing at 16.5° C.

Of the five tubers inoculated with each organism at each temperature, one was removed for the illustrations in Plates XIX and XX; the other four were cut and measured after 10 days. These measurements are shown in table 2. Where the rot was still active at the end of 10 days the figures are shown in bold face. The turgid cortex about a rotted pith which has been mentioned by Shapovalov and Edson (17) as characteristic of blackleg tuber rot and by Lacey (9) of *B. carotovorus* was noticeable in this experiment at 29° in the tubers inoculated with *B. aroideae, B. carotovorus,* and *B. phytophthorus,* but was absent in the lot inoculated with *B. mesentericus.*

The effect of temperature on the development of these rots was tested further in March, 1926. The same methods were used as in the previous experiment but the range of temperature was extended to 2° C., and two varieties, Green Mountain and White Rural, were included. The test lots were all distributed to the proper incubation chambers the day before inoculation to allow them to reach the desired temperature. They were then removed one lot at a time for inoculation and replaced at once. The test was concluded after seven days at the temperatures 15° to 33°, but was left for

TABLE 2.—*Effect of temperature on development of bacterial tuber rots in White Rural potatoes.* [*Bold face figures indicate that the rot was still active at the end of test.*]

Temperature in degrees C.	Relative humidity, per cent.	Average diameter of rot in mm. in 4 tuber samples after 10 days								Check 5 tubers
		B. mesentericus		*B. carotovorus*		*B. phytophthorus*		*B. aroideae*		
		Surface	1 cm. deep	Surface	1 cm. deep	Surface	1 cm. deep	Surface	1 cm. deep	
8	90	0	0	**11**	0	**24**	**13**	7	0	Sound
12	94	7	0	13	6	**20**	**21**	8	6	do
15.5	93	11	0	10	5	**23**	**27**	**11**	**22**	do
16.5	93	13	3	11	8	**26**	**25**	**14**	**18**	do
21	90	**13**	**20**	**13**	**15**	**19**	**25**	**16**	**24**	do
25	91	**21**	**30**	**14**	**18**	**24**	**31**	**20**	**31**	do
29	73	**38**	**41**	**16**	**30**	**15**	**29**	**29**	**38**	do
33.5	91	**40**	**41**	9	9	3	10	**40**	**47**	do

42 days at the lower range of 2° to 11° C. The results for both varieties are shown in table 3.

Marked differences in the effect of temperature on progress of the four types of rot appear from the data in tables 2 and 3. *Bacillus phytophthorus* invaded tubers at 2°–33.5° C. and proved destructive at 5.5°–29° C.; *B. carotovorus* attacked potatoes at 8°–33.5°, but was of little importance outside a narrow range between about 21° and 29°; *B. aroideae* was active from 11° up, and particularly virulent from 20° up; and *B. mesentericus* was even more distinctly a high temperature form, invading but feebly at 12°–16.5°, but a vigorous pathogen above 19° C. Both the high temperature forms were able to advance unchecked at favorable temperatures.

Few tests of the behavior of tuber rot bacteria at different temperatures have been published. Paine (14) reported that blackleg rot was often corked out of potato slices at 12°–14° C., but decomposed the whole slice at 20° C. Paine and Chaudhuri (15) in a study of *B. solanisaprus* and *B. atrosepticus*, both blackleg organisms and regarded as synonymous by some authors (5, 13), found the latter failed to infect at 30° but the former produced slight rot. Their table shows a steadily increasing amount of rot produced on slices at 12°–33° C. The results presented here show a less important effect of temperature on the activity of the blackleg organism, possibly due to different relations of cork formation in whole tubers as compared to slices. No previous study of the temperature relations of the other species of bacteria on potato has been seen by the writer.

The apparent decline in activity of *B. carotovorus* and particularly *B. phytophthorus* at the higher temperatures and the increasing activity

TABLE 3.—*Effect of temperature on development of bacterial tuber rots in White Rural and Green Mountain potatoes. Incubation period 7 days at 15° and above, 42 days at 11° and below.* [*Bold face figures indicate that the rot was still active at the end of the test.*]

Temperature in degrees C.	Relative humidity per cent.	Average diam. of rot in mm. in 5 tuber samples								
		White Rural								
		B. mesentericus		*B. carotovorus*		*B. phytophthorus*		*B. aroidae*		Check
		Surface	1 cm.	Surface	1 cm.	Surface	1 cm.	Surface	1 cm.	
2	99	0	0	0	0	0	0	0	0	Sound
5.5	98	0	0	0	0	**8**	**5**	0	0	do
8	93	0	0	0	0	**8**	**8**	0	0	do
11	97	0	0	0	0	11	6	11	4	do
15.5	94	7	0	6	0	8	6	**18**	**13**	do
19	95	**16**	**5**	5	4	**9**	**9**	**17**	**16**	do
22.5	90	**24**	**23**	11	10	10	4	**30**	**28**	do
28.5	92	**46**	**39**	**21**	**24.**	0	0	**48**	**47**	do
33	95	**56**	**47**	Contam.		Contam.		**63**	**63**	Contam.
		Green Mountain								
2	99	0	0	0	0	9	5	0	0	Sound
5.5	98	0	0	0	0	11	5	0	0	do
8	93	0	0	0	0	12	6	0	0	do
11	97	0	0	0	0	5	5	3	7	do
15.5	94	7	0	8	0	9	5	11	6	do
19	95	12	trace	9	0	7	4	10	8	do
22.5	90	**25**	**12**	0	0	6	5	**16**	**14**	do
28.5	92	**34**	**29**	6	7	4	4	**39**	**42**	do
33	95	**49** (3 contam.)	**32**	13 (1 contam.)	4	0 (2 contam.)	0	**60** (3 contam.)	**49**	3 sound, 2 contam.

of the other two species suggested tests at a still higher range. Four half tubers inoculated with a given culture and four corresponding halves pricked with a sterile needle were placed in covered glass dishes at six temperatures. At 25° and 30.5° typical rot was produced by all four organisms while the check tubers remained sound. At 37° the checks remained sound and the high temperature forms, *B. aroideae* and *B. mesentericus*, developed normal rot. *B. carotovorus* showed a trace of rot. *B. phytophthorus* proved clearly negative in one test at this temperature, but in a second trial the tissues about the inoculation point were blackened as in incipient rot. At 39° C. tubers inoculated with *B. phytophthorus* and

B. carotovorus remained sound except for slight local staining; the checks also were sound. *B. aroideae* and *B. mesentericus* invaded rapidly at 39°, with typical symptoms, but uninoculated checks frequently became contaminated. Both these organisms were reisolated from inoculated half tubers held at 39° and *B. mesentericus* was isolated from an uninoculated check half tuber incubated adjacent to potatoes inoculated with *B. mesentericus*. At 42° *B. mesentericus* rot was still typical in appearance and odor but the status of *B. aroideae* has not been clearly determined. Rot followed inoculation with this organism at 42° but uninoculated tubers also decomposed with a mixed population of organisms present. Tubers held in covered glass dishes at 49° C. showed heat necrosis throughout in 48 hours so that no index of parasitism was available. It appears that *B. mesentericus* rots potatoes vigorously at 20° C. and up to temperatures that are fatal to the tubers; *B. aroideae* is an active parasite as high as 39° C., possibly higher; *B. carotovorus* and *B. phytophthorus* are of minor importance, if any, at temperatures of 37° C. and above.

The four bacterial pathogenes considered fall into two fairly definite groups. *B. aroideae* and *B. mesentericus* are rapidly destructive from 20° C. to temperatures in excess of 35° C. *B. carotovorus* and *B. phytophthorus* have lower temperature requirements, which make them, especially the latter, dangerous to potatoes in cool storage. The two high temperature forms are of great potential importance to tubers which become heated in transit or in poor storage. *B. aroideae* in particular may be found destructive under field conditions also, when a survey of soft rots in the field is undertaken.

EFFECT OF HUMIDITY ON BACTERIAL TUBER ROTS

In the preliminary pathogenicity tests tubers were supported over water in 1-gallon crocks with loose-fitting covers and incubated at room temperature. Inoculation was made by needle prick, introducing young agar cultures through a freshly cut surface. This method afforded satisfactory results in the form of typical rot and sound checks. When similar inoculum was smeared on a cut surface rather than pricked into it, uniformly successful infection resulted with the virulent organisms, but the extent of rot was slightly less. When either smear or needle prick inoculations were made and the potatoes incubated at room temperature in crocks without water, infection was distinctly restricted. *B. aroideae*, *B. carotovorus*, and *B. phytophthorus* made no advance from smears, and their slight progress from the needle pricks was corked out. *B. mesentericus* made slight progress from the smears, which was stopped in all cases; in the needle pricks invasion took place in all cases but was blocked in four out of six.

TABLE 4.—*Effect of humidity at 12° and 22° C. on progress of tuber rots caused by 4 species of bacteria. Number of tubers showing infection and average diameter of rot in mm. at surface and at 1 cm. depth in 5 tuber samples. Irish Cobbler, inoculated April 21, measured May 10, 1927.* [*Bold face figures indicate that the rot was still active at close of experiment.*]

Inoculum	Tuber area observed	12° C.								22° C.							
		Relative humidity in per cent															
		87		74		67		55		94		90		82		78	
		No.	Diam.	No.	Diam.	No.	Diam.	No.	Diam.	No.	Diam.	No.	Diam.	No.	Diam.	No.	Diam.
B. mesentericus	Surface	5	7	0	...	1	6	0	...	5	14	5	10	5	**11**	5	8
	1 cm. deep	0	...	0	...	0	...	0	...	5	5	5	4	5	**7**	5	3
B. carotovorus	Surface	5	7	0	...	0	...	0	...	2	9	2	6	2	5	0	...
	1 cm. deep	0	...	0	...	0	...	0	...	1	2	0	...	1	2	0	...
B. phytophthorus	Surface	5	**21**	5	20	5	16	5	16	5	**21**	5	**18**	5	12	5	8
	1 cm. deep	5	**12**	5	10	5	9	5	11	5	9	5	**10**	5	6	5	6
B. aroideae	Surface	1	12	5	6	1	6	1	6	5	11	5	7	3	5	5	8
	1 cm. deep	1	5	2	6	1	4	0	...	5	3	5	3	5	3	4	3

To study more precisely the effect of humidity on the progress of these rots, the control chambers mentioned previously were again utilized. A series of four controlled humidities at 12° and a similar series at 22° C. were available. Each of the four bacterial pathogenes was inoculated into five Irish Cobbler tubers by needle pricks through a freshly cut surface, and these were placed together with five similar tubers pricked with a sterile needle in each of the eight control chambers. Fluctuations in control during this experiment were less than 2° C. in temperature and 5 per cent in humidity except that the two low humidities at 12° varied slightly more. The results of the test are summarized in table 4.

B. carotovorus produced negligible infection throughout and in all cases was stopped at the end of the 19-day period. *B. aroideae* produced little infection at 12°. At 22° this organism initiated rot in a majority of inoculated tubers, but was no longer active in any at the end of the test. *B. mesentericus* failed almost completely at 12°, but entered in all cases at 22° and remained active at 80 per cent humidity. *B. phytophthorus* was the most consistently successful invader throughout the test, and proved vigorous at the lowest humidity employed at 12°, but was inhibited by low humidity at 22° C.

Considering the method of inoculation employed in this test, low humidity could not be expected to prevent infection by a virulent pathogene inasmuch as the bacteria were forced deep into the moist tissues of the potato and could not become subject to drying for some time after inoculation. Neglecting the weak pathogene *B. carotovorus* and the performance of *B. mesentericus* at 12°, which is below the range of activity for this species, no effect of humidity on number of infections was evident. Diameter of penetration, on the other hand, is a measure of the ability of the parasite to continue activity under the test conditions. Here a general tendency appeared toward diminished penetration with lower humidity, a trend which applied to all four organisms. The few cases in which rot remained active at the end of the test were at humidities above 80 per cent. The general indication is that low humidities restricted advance of these rots, but failed to check completely the advance of a virulent pathogene in a deep wound.

REACTION OF POTATO VARIETIES TO BACTERIAL TUBER ROTS

No previous study of the reaction of potato varieties to soft rots other than blackleg rot has been seen by the writer. Work on varietal susceptibility to blackleg has been reviewed by Kotila and Coons (7). These authors conclude from their studies that no variety is resistant enough to preclude loss, and that tuber reaction is not correlated with stem susceptibility. On

the basis of tuber susceptibility they list the following varieties in order, beginning with most resistant: Triumph, Green Mountain, Russet Rural, Irish Cobbler, Early Ohio, Sir Walter Raleigh, Carman No. 3.

In the present work ten varieties were tested at least once, some three times, for susceptibility to four bacterial rots. For the first and second tests run in January, 1926, the lots of Cobbler, Russet Rural, White Rural, and mature Green Mountain were grown in one field in Pennsylvania and were strictly comparable; Spaulding Rose came from another section of Pennsylvania, Triumph from Maine, McCormick and immature Green Mountain from Arlington Farm, Va. The third test, run a year later, included Cobbler, Russet Rural, Green Mountain, Spaulding Rose, Triumph, and Early Rose from one field in Pennsylvania, Early Ohio and Burbank from Maine, and McCormick from Arlington Farm. A stock of Green Mountain grown in Vermont in 1925 and stored about 18 months at 4.5° C. was added. Inoculation in the first and third tests was made by pricking a young agar culture into the tubers through a freshly cut surface to a depth of about 3 cm. by means of a heavy platinum needle. In the second test 1 cc. of a 3-day broth culture was poured into a well 8 mm. in diameter and from 2 to 3 cm. deep in each tuber. The wells were not sealed in any way. The use of wells instead of needle wounds tended to magnify the diameter of the rot in this second test. The inoculated tubers in the first and second tests were supported over water in loosely covered 1-gallon crocks and incubated 16 days at room temperature; in the third experiment they were incubated 11 days in 4-quart baskets at 21° C. and approximately 80 per cent humidity. Because of these differences in method of inoculation and incubation the three tests were not exactly comparable, but all varieties in any one test were treated alike. Check lots of 5 tubers of each variety were included in each test; of 130 such tubers all but one remained sound.

In table 5 the varieties are listed in order of decreasing susceptibility according to their average performance with respect to all four organisms in one or more of the three experiments. These data show that the varieties Burbank, Early Ohio, Spaulding Rose, and the lot of Green Mountain from prolonged storage were more susceptible to blackleg rot than to the other three types of decay. With these exceptions the reactions of the several varieties to the four types of rot are in general of the same order. Russet Rural and White Rural were clearly most susceptible. Russet Rural alone afforded all four bacteria suitable conditions for continued rot at the comparatively low humidity used in the third test, and Russet Rural and White Rural showed less ability to block out the rots than the other varieties tested. *B. mesentericus, B. aroideae,* and *B. phytophthorus* were all active at the conclusion of each test with the Rural types, and *B. carotovorus* remained

TABLE 5.—*Reaction of potato varieties to bacterial tuber rots.* [*Average diameter of rot in mm. in 5 tuber samples. Duration of first and second tests 16 days, third test 11 days. Bold face figures indicate that the rot was still active at close of experiments.*]

Variety	*B. mesentericus*			*B. carotovorus*			*B. phytophthorus*			*B. aroideae*			Average all organisms
	1st test	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	
White Rural	**27**	**25**	....	10	14	....	**29**	**17**	....	**32**	**45**	....	25
Russet Rural	**15**	**31**	**26**	9	14	**13**	**20**	**17**	**39**	**33**	**50**	**17**	24
Burbank	....	....	8	....	....	6	....	....	**41**	....	....	14	17
Irish Cobbler	**7**	**24**	8	**14**	0	2	**30**	**28**	**11**	**22**	**32**	6	15
Green Mountain, 18 months old	....	....	**9**	....	....	7	....	....	**36**	....	....	**22**	19
Green Mountain, immature	**19**	**18**	....	**15**	0	....	**5**	**10**	....	**13**	**35**	....	14
Green Mountain, mature	**4**	**16**	**10**	8	0	5	**7**	**26**	**10**	**9**	**35**	**9**	12
Triumph	**6**	**11**	**10**	4	0	6	**16**	**33**	**9**	**22**	**31**	**9**	13
Early Ohio	....	....	5	....	....	5	....	....	**22**	....	....	8	10
Early Rose	....	....	8	....	....	5	....	....	**12**	....	....	**8**	8
Spaulding Rose	**2**	**0**	**7**	**0**	**0**	5	**3**	**30**	**10**	**4**	**0**	**9**	6
McCormick	**0**	**14**	**7**	**0**	**0**	4	**0**	**13**	**3**	**8**	**11**	**9**	6

active once. The Irish Cobbler was relatively susceptible to all, but only blackleg gained a firm foothold in this variety in the last and least favorable test. McCormick was most resistant; Spaulding Rose consistently warded off all but blackleg rot. Green Mountain and Triumph fell in the middle ground, less susceptible than the Rurals and Cobbler but much more so than Spaulding Rose and McCormick. Burbank, Early Ohio, and Early Rose could be placed only tentatively from a single test. Little difference appeared between stocks of Green Mountain harvested when immature (Arlington) and when mature (Pennsylvania), but the lot stored for 18 months showed increased susceptibility to all the organisms. Plates XXI and XXII show the appearance of two highly susceptible and two slightly susceptible varieties at the conclusion of the first experiment. In White Rural and Cobbler all infections have made good progress, but the *B. carotovorus* is no longer active in the Rural. In Spaulding Rose *B. carotovorus* has not progressed, *B. mesentericus* and *B. aroideae* have been stopped after slight progress, and *B. phytophthorus* is still alive. In McCormick, *B. aroideae* has made distinct advance and *B. mesentericus* a trace, but both are blocked out.

PATHOGENICITY OF TUBER ROT BACTERIA FOR OTHER HOSTS

Various plants were inoculated with each of the four organisms under study, particularly to determine whether *B. mesentericus* was capable of attacking plant parts other than potato tubers. The three soft-rot organisms were included to provide a measure for the effectiveness of the conditions of inoculation and incubation. From 2 to 15 plants were inoculated with each organism and a like number of uninoculated plants were held under comparable conditions in each test. Inoculations were made by pricking young agar cultures into the plants with a sterile needle. In laboratory tests the inoculated plant parts, except the cabbage heads, were supported over water in covered crocks and incubated at room temperature. The cabbage heads were treated similarly, but no water was put in the crocks. In the greenhouse experiments the plants were incubated for from 2 to 3 days in a glass-walled moist compartment, or were covered with bell jars for a like interval, then returned to the open bench. The greenhouse temperatures usually ranged from 15 to 20° C. at night and were often higher during the day. The results of all tests are summarized in table 6. It will be seen that *B. mesentericus* proved uniformly negative on plants other than the potato, and on parts of the potato plant other than the tubers. Two doubtful cases and other observations of interest will be discussed further.

Laboratory Experiments: Carrot roots. Bacillus carotovorus, B. aroideae, and *B. phytophthorus* all caused marked decay of carrots in four

days. No consistent difference in symptoms was observed, but B. phytophthorus rot was in some cases darker in color. B. aroideae rot progressed most rapidly. Needle inoculations with *B. mesentericus* were without effect, but when a young broth culture of this organism was poured into cork borer wells in carrots, one root was invaded for 2–5 mm., two others remaining sound.

Turnip roots. The three soft-rot organisms, *B. carotovorus, B. aroideae,* and *B. phytophthorus* caused an active brown soft rot of turnips in four days.

Cabbage heads. Cabbage heads were rotted by the soft-rot bacteria to a diameter of 10–15 mm. along the whole depth of the needle pricks in four days. At the end of this period *B. carotovorus* and *B. phytophthorus* were still active, but the B. aroideae rot had dried out.

Iris versicolor. Plants of the variety Queen of May were brought into the greenhouse in March, and inoculated into the young rootstocks and at the base of the young leaves, then incubated in crocks in the laboratory. Both rootstocks and leaves were attacked by all three of the soft-rot bacteria. In five days B. carotovorus rot in the rootstock had dried out, *B. phytophthorus* had half destroyed the rootstock, and *B. aroideae* completely destroyed it. In young leaf bases all three caused rapid translucent watery decay, which spread upward into the leaves 2–9 cm. and brought about complete collapse of the plants.

Greenhouse Experiments: Potato stems. In the spring of 1925 cultures of the blackleg organism and of *B. carotovorus* and *B. mesentericus* were inoculated into young stems of Russet Rural potatoes. At the end of seven days the blackleg organism had killed three plants completely and a fourth was killed above the needle wound but was sprouting below. *B. carotovorus* killed one stalk but two others healed without appreciable injury. Of the two inoculations with *B. mesentericus,* one brought complete collapse, the other killed the shoot above the lesion but growth was resumed below. Two check plants pricked with a sterile needle remained sound.

This experiment was repeated twice in 1926, with *B. aroideae* added. At the end of three days' incubation under bell jars, *B. phytophthorus, B. aroideae,* and *B. carotovorus* had completely killed three Russet plants inoculated with each, but three plants inoculated with *B. mesentericus* and three check plants remained entirely healthy. Irish Cobbler stems reacted similarly. Two plants inoculated with *B. phytophthorus* and three with B. *aroideae* were killed in six days; *B. carotovorus* killed one, and half girdled a second, but three shoots pricked with *B. mesentericus* and one check plant remained sound. The three species producing active stem rot were indistinguishable in behavior on potato stems. The discoloration

produced by the blackleg organism was occasionally but not consistently darker than that caused by *B. aroideae* and *B. carotovorus*, but all three blackened the tissues.

Tomato stems. In six days *B. aroideae* and *B. phytophthorus* each killed two of three tomato plants inoculated, and produced local lesions which dried out in the third. *B. carotovorus* formed small local lesions which dried out without appreciable injury.

Bean stems. Nine bush bean plants were inoculated with each of four organisms. At the end of 10 days four plants inoculated with *B. carotovorus*, three with *B. phytophthorus*, and three with *B. aroideae* had softened at the needle prick and collapsed. Some of the remaining plants inoculated with each of these parasites showed local browning and cracking at the wound, but the *B. mesentericus* and check sets remained healthy.

Cucumber seedling stems. Fifteen cucumber seedlings in the two-leaf stage were inoculated about 2 cm. above the soil level with each of the four organisms, and fifteen were held as checks. In six days *B. aroideae* had softened the stems of nine plants and brought about complete collapse at the point of inoculation. No evidence of invasion by *B. carotovorus*, *B. phytophthorus* or *B. mesentericus* was detected. One check plant damped off at the soil line.

Cabbage seedling stems. Cabbage seedlings about 10 cm. tall had completely collapsed upon removal from the moist chamber two days after inoculation with *B. aroideae*, *B. carotovorus*, and *B. phytophthorus*. A translucent, watery decay extended up the stem into the leaf petioles and veins in all cases, and well into the leaf lamina in the case of *B. carotovorus* and *B. aroideae*. The plants inoculated with *B. mesentericus* and the check plants remained healthy.

Pelargonium zonale stem cuttings. Two well-rooted cuttings with five or six leaves were inoculated with each culture. In five days *B. carotovorus* killed one but did not injure the other; *B. phytophthorus* killed one and produced a local lesion on the second; *B. aroideae* killed one completely and killed the bud of the other. Plants inoculated with *B. mensentericus* remained sound; one of two check plants was killed by Botrytis infection present before incubation.

Iris versicolor leaves. Iris plants brought from an outdoor planting to the greenhouse in March were inoculated in June. When the plants were removed from the moist chamber after three days, the three soft-rot bacteria, *B. aroideae*, *B. phytophthorus*, and *B. carotovorus*, had produced marked decay, a watery zone extending upward into the leaves from the needle wound for 2–25 cm. One of the checks rotted; two others and the plants inoculated with *B. mesentericus* remained healthy. These results

are not in accord with Massey's (12) report that iris is a differential host for *B. aroideae* and *B. carotovorus*, the latter but not the former attacking it.

TABLE 6.—*Summary of inoculation tests with four bacterial pathogenes on various hosts*

Host	Part inoculated	*Bacillus mesentericus*	*B. carotovorus*	*B. phytophthorus*	*B. aroideae*	Checks
		Laboratory tests				
Carrot	Root	0 ?	+	+	+	Sound
Turnip	do	0	+	+	+	do
Cabbage	Head	0	+	+	+	do
Iris versicolor	Root stock and leaf bases	0	+	+	+	do
		Greenhouse tests				
Potato	Stems	0 ?	+	+	+	do
Tomato	do	0	Trace ?	+	+	do
Bean	do	0	+	+	+	do
Cucumber	Seedling stem	0	0 ?	0 ?	+	14 sound 1 damped off
Cabbage	do	0	+	+	+	Sound
Pelargonium zonale	Stem cutting	0	+	+	+	1 sound 1 Botrytis
Iris versicolor	Base of leaf	0	+	+	+	2 sound 1 rotted
Zantedeschia aethiopica	Petioles	0	0	0	+	Sound

Zantedeschia aethiopica petioles. Petioles of the white calla lily were inoculated repeatedly with the four organisms under study but only *B. aroideae* produced any effect on this host. When petioles were inoculated with this organism, slight brown watery lesions appeared at the needle wound in three days and gradually increased in size. In seven to nine days blotches appeared on the leaf blades and the leaves turned yellow and collapsed. In some instances the rot spread to other leaves of the same plant and these collapsed in turn. Inoculation of this host, as already pointed out by Massey (12), is apparently a reliable means of distinguishing *B. aroideae* from *B. carotovorus*. This point should be tested further with additional strains, especially inasmuch as Bewley (2) has reported

B. carotovorus and not *B. aroideae* as the cause of soft rot of the calla lily in England.

SUMMARY

Bacillus mesentericus was isolated from a wound rot of potato tubers. Pure cultures of this organism readily produced rot in healthy potatoes at 20° C. and above. The pathogenicity of cultures was retained after heating to 80° C. for 15 minutes.

Bacillus mesentericus was found to grow on artificial media within the range 8–49° C. with an optimum at 39–42° C. Three other species pathogenic to tubers, *B. aroideae, B. carotovorus,* and *B. phytophthorus,* showed lower minima, optima, and maxima.

Bacillus mesentericus invaded potato tubers feebly at temperatures below 20° C., but advanced more rapidly as the temperature was increased, and showed no maximum within the range at which potatoes could be held without injury. *B. aroideae* showed a similar temperature curve, with a slightly lower minimum, and greater virulence. These two may be grouped as potentially dangerous to potatoes improperly stored, or heated in transit. *B. carotovorus* and *B. phytophthorus,* particularly the latter, showed lower minima for growth on raw potato, and also a tendency to reach a maximum near 30° C., above which they declined in activity.

At the temperatures 12° and 22° C. relative humidity had no effect on the number of infections resulting from needle inoculations into the interior of tubers, but relative humidity lower than 80 per cent checked the progress of all four bacteria at these temperatures.

The varieties Rural New Yorker and Russet Rural, followed by Irish Cobbler, were most susceptible to all four types of rot. McCormick was most resistant, and Spaulding Rose was resistant to all but blackleg rot. Five other varieties are classed between these extremes on the basis of limited data.

Bacillus mesentericus did not attack other plants or other parts of the potato than the tuber.

OFFICE OF VEGETABLE AND FORAGE DISEASES,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

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EXPLANATION OF PLATES

PLATE XIX.

Progress of bacterial rots in White Rural potatoes at eight temperatures. Photographed three days after inoculation. Left: *Bacillus mesentericus;* right *B. aroideae.* The actual average temperatures during incubation were, reading from top to bottom, left column: 8°, 12°, 15.5°, 16.5° C.; right column: 21°, 25°, 29°, 33.5° C.

PLATE XX.

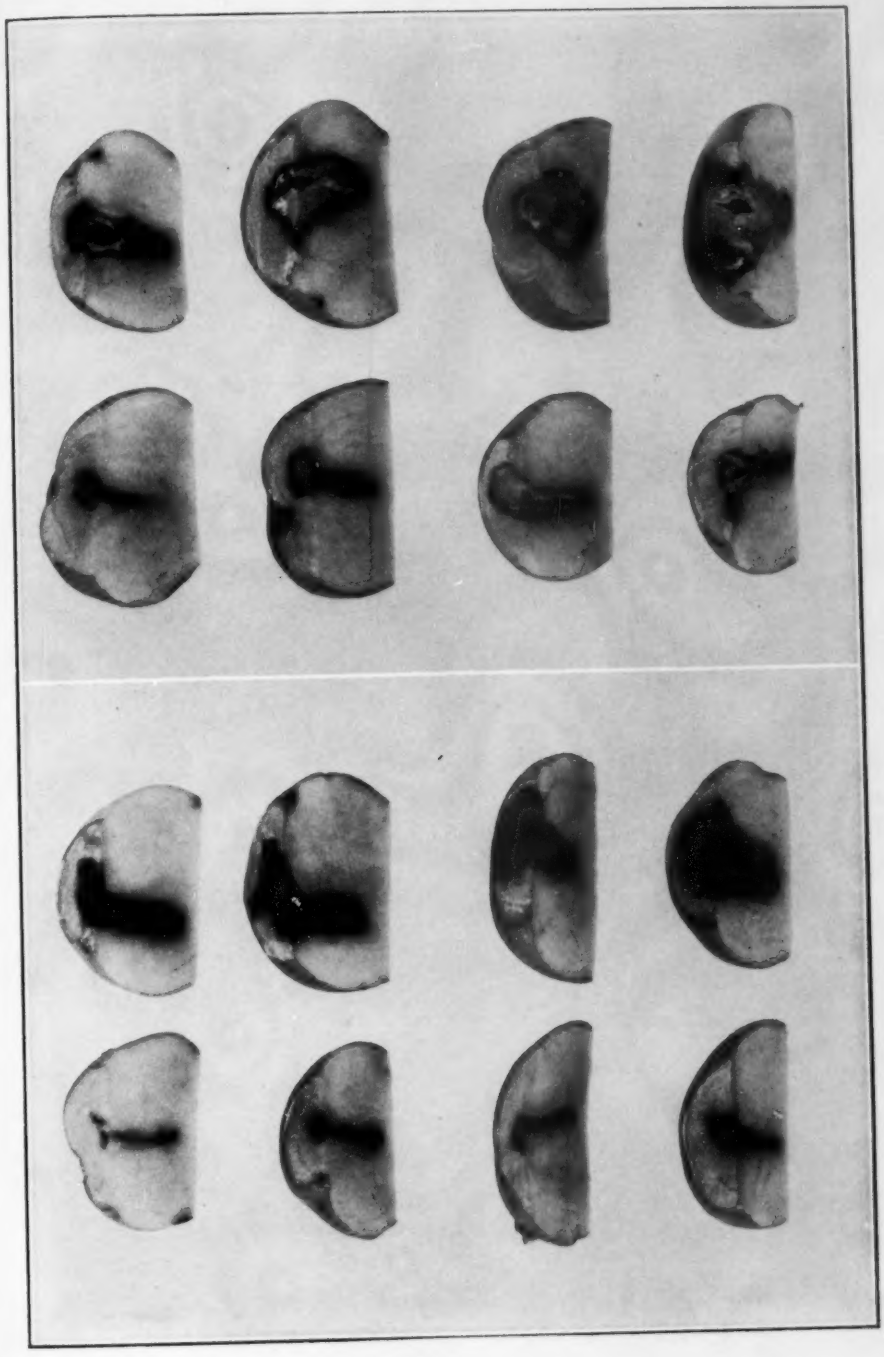
Progress of bacterial rots in White Rural potatoes at eight temperatures. Photographed three days after inoculation. Left: *Bacillus carotovorus;* right: *B. phytophthorus.* The actual average temperatures during incubation were, reading from top to bottom, left column: 8°, 12°, 15.5°, 16.5° C.; right column: 21°, 25°, 29°, 33.5° C.

PLATE XXI.

Reaction of potato varieties to bacterial rots. Inoculated January 5, photographed January 21, 1926. Incubated at room temperature. Left: White Rural; right: Irish Cobbler. CK. pricked with sterile needle, 75 *Bacillus mesentericus,* 475 *B. carotovorus,* 526 *B. phytophthorus,* 552 *B. aroideae.*

PLATE XXII.

Reaction of potato varieties to bacterial rots. Inoculated January 5, photographed January 21, 1926. Incubated at room temperature. Left: Spaulding Rose; right: McCormick. CK. pricked with sterile needle, 75 *Bacillus mesentericus,* 475 *B. carotovorus,* 526 *B. phytophthorus,* 552 *B. aroideae.*



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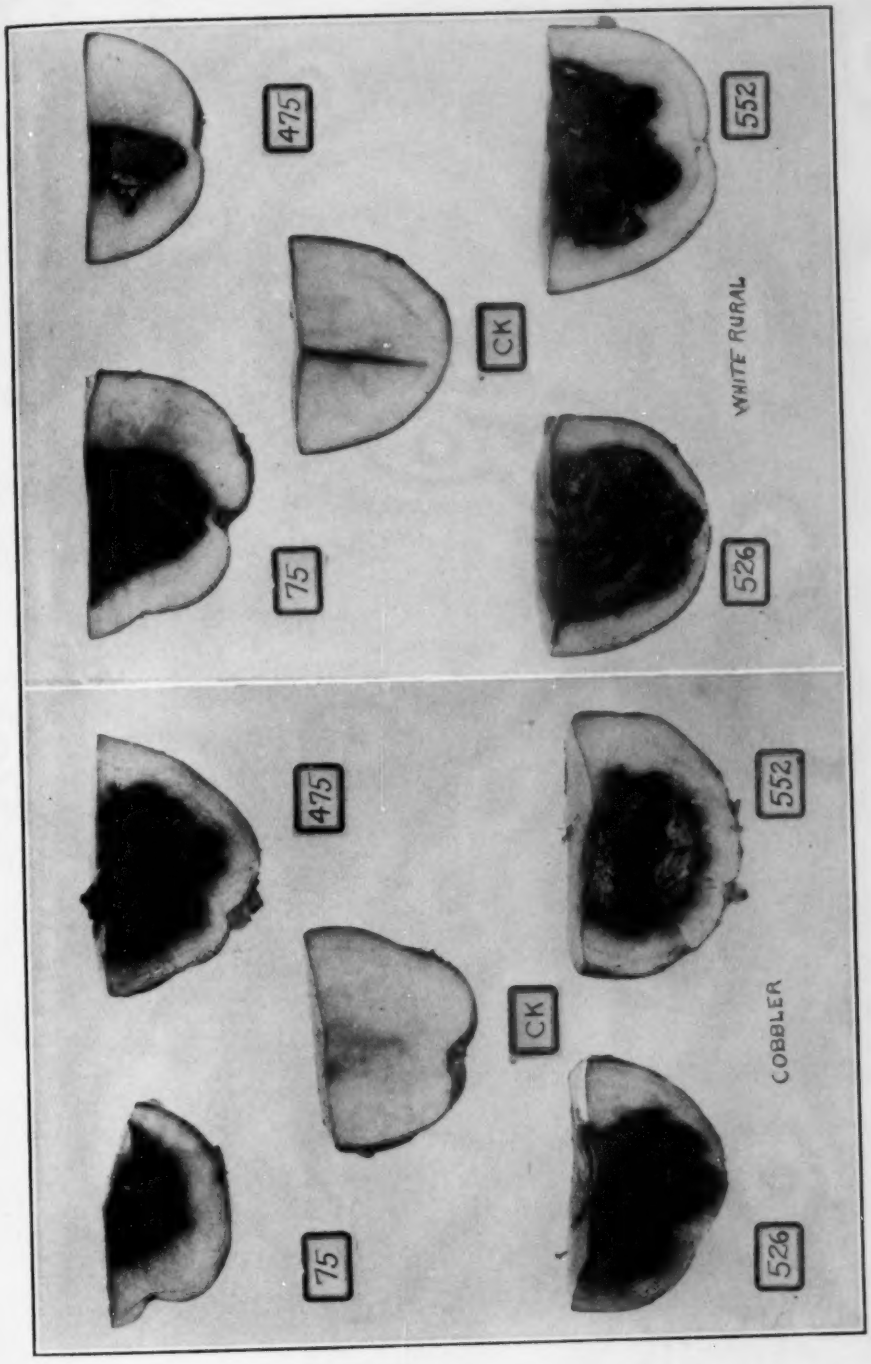

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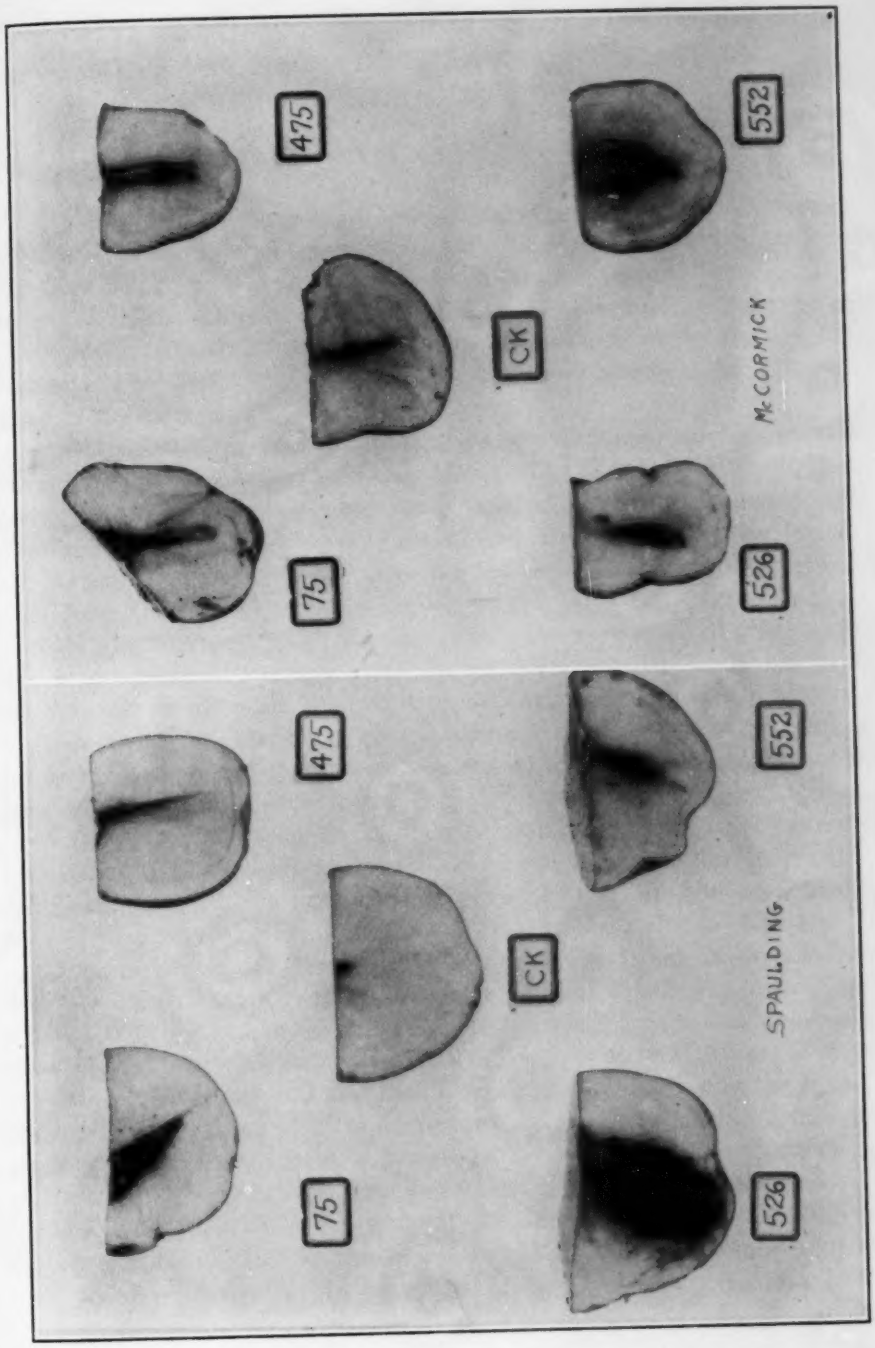

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PRELIMINARY STUDIES OF THE LIFE HISTORY OF EROSTROTHECA MULTIFORMIS, THE PERFECT STAGE OF CLADOSPORIUM ALBUM DOWSON

G. Hamilton Martin and Vera K. Charles[1]

In April, 1927, specimens of diseased sweet peas (*Lathyrus odoratus*) were received for determination by the Office of Mycology and Disease Survey from Dr. E. F. Guba, of the Massachusetts Experiment Station. Examination of the material showed the disease to be caused by a species of *Cladosporium* identical with *Cladosporium album* described by Dowson (4) in England in 1924. "White blight" is suggested by the authors for the name of the disease.

In May, 1927, the senior author made a preliminary survey for this disease in the greenhouses on Long Island, N. Y., and in northern New Jersey, where sweet peas were being grown. During this survey the disease was observed in only one greenhouse, that being on Long Island, although later in the year two more reports with specimens were received from New York.

Early in February, 1928, diseased sweet peas were received from a section in southeastern Pennsylvania. In all cases reported, the disease was the cause of considerable loss. One grower had an entire crop destroyed, while another grower who had been shipping to the New York City market for over 15 years said that unless a means of control was found he would have to stop growing sweet peas.

A critical examination of the Massachusetts, New York, and Pennsylvania collections showed the casual organisms to be identical with each other and also with the type material received from Mr. Dowson and deposited in the Mycological Collections of the Bureau of Plant Industry.

Cultures were made from the Massachusetts and New York material with the result that a perfect stage was developed from the New York collection. From this latter material only 1 out of 15 parts and vigorously growing cultures produced perithecia. The fact that Dowson failed to obtain a perfect stage and also that it was not developed from the Massachusetts material can best be explained by assuming the presence of strains. Certain phenomena exhibited in the course of the cultural work, which will be discussed in a later paper, also support this hypothesis.

The most remarkable character of the fungus under consideration is its polymorphism. In addition to seven distinct stages, a pseudo-sclerotial form was also observed. In certain of these forms the transitions from

[1] The writers are under obligation to Mr. M. L. F. Foubert for the photographic work illustrating this paper.

one stage to another can be easily understood and interpreted from a single slide. This is especially true in young or vigorously growing cultures. In old cultures the forms are found to adhere more closely to the types of the form genera that they represent. Conditions of temperature and humidity, and kind of media used are found to have a very great influence on the particular type of spore produced, but in this preliminary report no attempt will be made to correlate the character of growth with the physiological conditions under which the fungus has been grown.

At the present time the authors are unable to give a formula for the sequence in the development of the different spore forms, if indeed there is any law controlling such development. The object here is to describe the different forms as they appear in nature and in pure cultures.

Considerable latitude may be permitted in definitely placing some of the conidial forms in the proper form genera. In view of the fact that these forms are all phases of the same species, the names used are more for convenience in describing them than for giving them a definite taxonomic position. The nomenclature followed is in most respects that of Briosi and Farneti (1) in their paper on white blight of lemon.

While the organism described in their paper is not identical with the present fungus, there are certain resemblances in the life histories of the two fungi which make their comparison of interest. A pycnidial stage was found by Briosi and Farneti and described as *Rhynchodiplodia,* but no ascospore stage was noted. On the contrary, in the study of the sweet pea fungus, no pycnidial stage was found but a perfect stage developed. The comparable conidial stages of the two fungi are as follows:

Rhynchodiplodia Briosi and Farneti	*Erostrotheca* Martin and Charles
CONIDIAL FORMS	CONIDIAL FORMS
Cladosporium citri	*Cladosporium album*
Hormodendron citri	*Hormodendron cladosporioides*
Ovularia citri	*Ovularia* form
Haplaria citri	*Haplaria* form
Pseudofumago citri	*Pseudofumago* form
Pseudosaccharomyces citri	*Pseudosaccharomyces* form
PYCNIDIAL FORM	PYCNIDIAL FORM
Rhynchodiplodia citri	Not found
SCLEROTIA	SCLEROTIA
Not given	*Pseudosclerotia*
ASCOGENOUS FORM	ASCOGENOUS FORM
Not given	*Erostrotheca multiformis*

DESCRIPTION OF THE DISEASE AND CAUSAL ORGANISM

The leaflets were covered with tan or buff-colored, circular and irregular-shaped spots varying from 2 to 20 mm. in diameter and often coalescing into large necrotic areas involving all or a large portion of the entire leaflet (Plate XXIII, A). The spots showed a tendency to be water-soaked about the margins. A close examination revealed numerous cinnamon-brown pustules, which imparted a granular appearance to the surface (Plate XXIII, B). On this infected area, numerous whitish tufts could be seen. These tufts consisted of conidiophores of the *Cladosporium-Hormodendron* form, and, when fresh, imparted to the spots a mealy appearance. In addition to this form, *Pseudofumago* and *Pseudosaccharomyces* were also present and became conspicuous when the leaves were allowed to remain in a moist chamber for 2 or 3 days.

The pseudosclerotia developed on the diseased areas in the course of a few days (Plate XXIII, C).

CULTURAL WORK

The inoculum for the culture work was all from the original culture of *Cladosporium album* in which the ascogenous stage developed. Accompanying morphological comparisons were made with the fungus on affected leaves received in February and March, 1928.

Various culture media were employed, but the larger part of the experimental work was done with cornmeal agar. It may be mentioned here that transfers from the same culture, when grown on potato and cornmeal agar, gave different types of growth. This applied not only to the production of conidial forms, but also to the development of perithecia, which were developed in abundance on cornmeal agar but not at all on potato agar.

For the sake of simplicity in treatment, the discussion will begin with the germination of the ascospore.

Ascospores (Plate XXIV, F) germinated (Plate XXIV, B and Plate XXV, A and B) readily at temperatures from 65° to 85° F. in about 18 hours. The growth was rapid and luxuriant, and conspicuously zonate. A *Haplaria* (Plate XXV, B and Plate XXVII, C) form was observed at the end of a few days. The *Cladosporium-Hormodendron* form of fructification followed in a short time. The *Cladosporium* consists of conidiophores bearing chains of 3–5 oblong, one- or two-celled hyaline spores (Plate XXIV, A, D). In old cultures the chains of spores were much longer, sometimes consisting of 10–12 spores. The usual form of *Hormodendron* appearing as a stage in the life history of this fungus and the only form observed by Dowson (4, p. 226) appears to differ from *Hormodendron cladosporioides* only in having larger hyaline conidia. In the course of these studies a dark form also developed from ascospore cultures.

The *Ovularia* form was observed at different periods in the growth of the cultures. In some cases it was found to develop from fine mycelium arising directly from the *Pseudofumago* and again it appeared in cultures of different ages. The spores are very small and often adhere to each other, forming an agglutinated head. The conidiophores are very erect, arise singly or in groups of 2 or 3, and measure about 28–35 μ in length. The spores are 1.5–2.5 × 3.5–4.5 μ. The size of the spores is very dependent on the character of the medium.

The earliest stages in the development of perithecia were observed when the cultures were about six days old (Plate XXV A, Plate XXIV, A and B). They were formed abundantly and grew rapidly, maturing in about three weeks. (Plate XXIV, C). The irregularly biseriate arrangement of the spores in the ascus may be seen at this time (Plate XXIV, E).

Pseudofumago occurred at different periods of growth and in association with the other spore forms. It consisted of coarse, irregular mycelium, composed of globular or ellipsoidal cells varying in size from 3–4 to 13–17 μ (Plate XXV, C). It was colorless when present in the tissues of the leaves. In culture the mycelium was hyaline, later becoming dark brown or greenish black. In this condition one or more cells may separate, becoming thick-walled and sometimes transversely divided. They are analagous to spores, capable of immediate germination or functioning as resting spores.

The Pseudosaccharomyces type of development was found to proceed directly from the *Pseudofumago*. This stage is so called on account of its resemblance to *Saccharomyces* and its manner of budding (Plate XXVI, A and B, and Plate XXVII, D). The various manifestations of growth exhibited during the transition of *Pseudofumago* and *Pseudosaccharomyces* are very numerous. *Pseudosaccharomyces* exhibits a remarkable polymorphism in its manner of budding and spore formation. Conidia may be borne in a whorl or crown at the apex of a terminal cell or on a protuberance which arises from any of the cells along the course of the mycelium. In advanced stages of the disease a pseudosclerotial form was observed on the leaves.

Forms of *Pseudosaccharomyces* are so similar to certain species of various form genera as to be indistinguishable from them morphologically. Among certain species included in this category may be mentioned *Protocoronospora nigricans* Atk. and Edgert. (10), *Kabatiella nigricans* (Atk. and Edgert.) Karak (6) on *Vicia*. All the budding conidial stages described and illustrated for this species correspond exactly with the species concerned in this investigation. It is of historical interest to note that in 1905 importations of *Vicia faba* originally from Egypt and Italy that were referred to this office were affected with apparently the same organism; in

this material, in addition to the budding form, several other stages comparable to those appearing in the present cultural work were noted. The fungus was not definitely determined at that time, it being two years previous to the publication of *Protocoronospora*. The similarity was confined to the conidial stages, no perfect form being found.

In April, 1927, various species of lilies growing in the vicinity of Washington were found to be affected with a disease that in morphological and cultural characters corresponds with the budding stages of the present fungus. Old cultures developed a *Pseudofumago* comparable to the *Pseudofumago* form discussed here. This fungus was identified as *Kabatiella*, probably *K. microsticta* Bub. described by Bubak (2) in 1907 as occurring on living leaves of the lily-of-the-valley in Bohemia. *Polyspora lini* (7) also presents comparable budding stages.

An attempt was made to place the perfect stage of the sweet pea fungus in a genus already established, but careful adherence to the generic description of nearly related genera made this impossible.

The fact that the spores are colored necessitated placing the genus in the group Phaeosporae of the Hypocreaceae.

In this section the genus *Melanospora* (3) is separated by the development of the beak and presence of dark spores. In *Neurospora* (8) the perithecia are described as subcoriaceous to subcarbonous, and the spores black when mature and longitudinally ribbed. A further separation is seen in the development of the perithecia. At no time in the development of the present fungus is the perithecial cavity filled with crowded septate hyphae such as are described for *Neurospora*. While the genus *Sphaeroderma* (5) is described as beakless, and the subiculum in some species is only poorly developed, it has several points of difference, among which are the longer-stiped, 4-spored asci; the spores, too, are characteristically darker than in the fungus described here. Even certain species of the genus *Sphaeroderma* described with 8-spored asci differ in other respects from the fungus described in this paper.

While there is a macroscopic resemblance to *Neocosmospora* (9) in the bright color of the perithecia and in the character of the walls, the genus differs in possessing brown, globose, verruculose spores and further by the absence of paraphyses.

Erostrotheca gen. nov.

Perithecia superficial, gregarious or scattered, globose-conical, beakless, glabrous, diaphanous; stroma absent; asci arising basally, evanescent, 8-spored; spores ellipsoid, yellow to olivaceous. (Perithecia superficial or embedded when grown in media.)

Erostrotheca multiformis sp. nov.

Perithecial stage; perithecia gregarious or scattered, characteristically zonate, when grown on cornmeal agar, at first Capucine yellow changing to mahogany red with age; asci clavate, 20–25 × 12–16 μ, aparaphysate, short stipitate; ascospores irregularly biseriate, elliptical, flattened on one side, dark citrine, olive-yellow in mass. In agar culture.

Conidial stages: (*Cladosporium album* Dowson) spores elongate-cylindrical, continuous or 1-septate, 14–18 × 3–4 μ: *Hormodendron* form, chains of spores simple or branched; spores pip-shaped to elliptical, both hyaline and colored in ascosporic cultures. *Ovularia* form, conidiophores erect, arising singly or in groups of 2 or 3, 28–35 μ in length; spores 1.5–2.5 × 3.5–4.5 μ, often adhering to form agglutinated heads. *Haplaria* form, hyphae branched, hyaline, septate; conidiophores papillose or stipitate; conidia elliptical, continuous, hyaline, 6–8 × 2–4 μ, forming agglutinated groups. *Pseudofumago* form, coarse, irregular mycelium, consisting of globular or irregular cells, 3–4 × 13–17 μ. *Pseudosaccharomyces* form, cells saccharomycetous, hyaline, 8–16 × 4–8 μ; conidia acrogenous or pleurogenous, spherical to oblong-elliptical. Pseudosclerotia minute brown to black on diseased areas of leaves.

Habitat: On living leaves and stems of *Lathyrus odoratus.*

SUMMARY

A recently recorded sweet pea disease in America caused by a *Cladosporium* is shown to be identical with *C. album* Dowson. This disease was described by Dowson in England in 1924 and recorded in America in 1927. "White blight" is suggested as the common name for this disease.

The perfect stage of *Cladosporium album* was developed in culture media and is described as *Erostrotheca multiformis.*

The fungus has many different spore forms, each of which is capable of starting a new infection. These are forms of *Cladosporium, Hormodendron, Haplaria, Ovularia, Pseudofumago,* and *Pseudosaccharomyces.* Pseudosclerotia are formed on the necrotic areas of leaves and are a means of spreading the disease.

Habitat: On leaves and stems of *Lathyrus odoratus.*

Known distribution: England, Massachusetts, New York, and Pennsylvania.

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A list of ten references is added for the use of workers especially interested in this subject.

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EXPLANATION OF PLATES

PLATE XXIII.

A. Sweet pea leaflets showing spots caused by *Erostrotheca multiformis*.
B. Portion of diseased leaflet showing granular appearance of spot. ×2.
C. Pseudosclerotia formed on diseased leaflet. ×2.

PLATE XXIV.

A. Conidiophore and spores of *Cladosporium*. ×400.
B. Germinating ascospores. ×400.
C. Mature perithecia. ×about 168.
D. Young culture from a single ascospore showing conidiophore and spores of *Cladosporium* and young perithecium. ×400.
E. Ascus with spores. ×400.
F. Ascospores. ×400.

PLATE XXV.

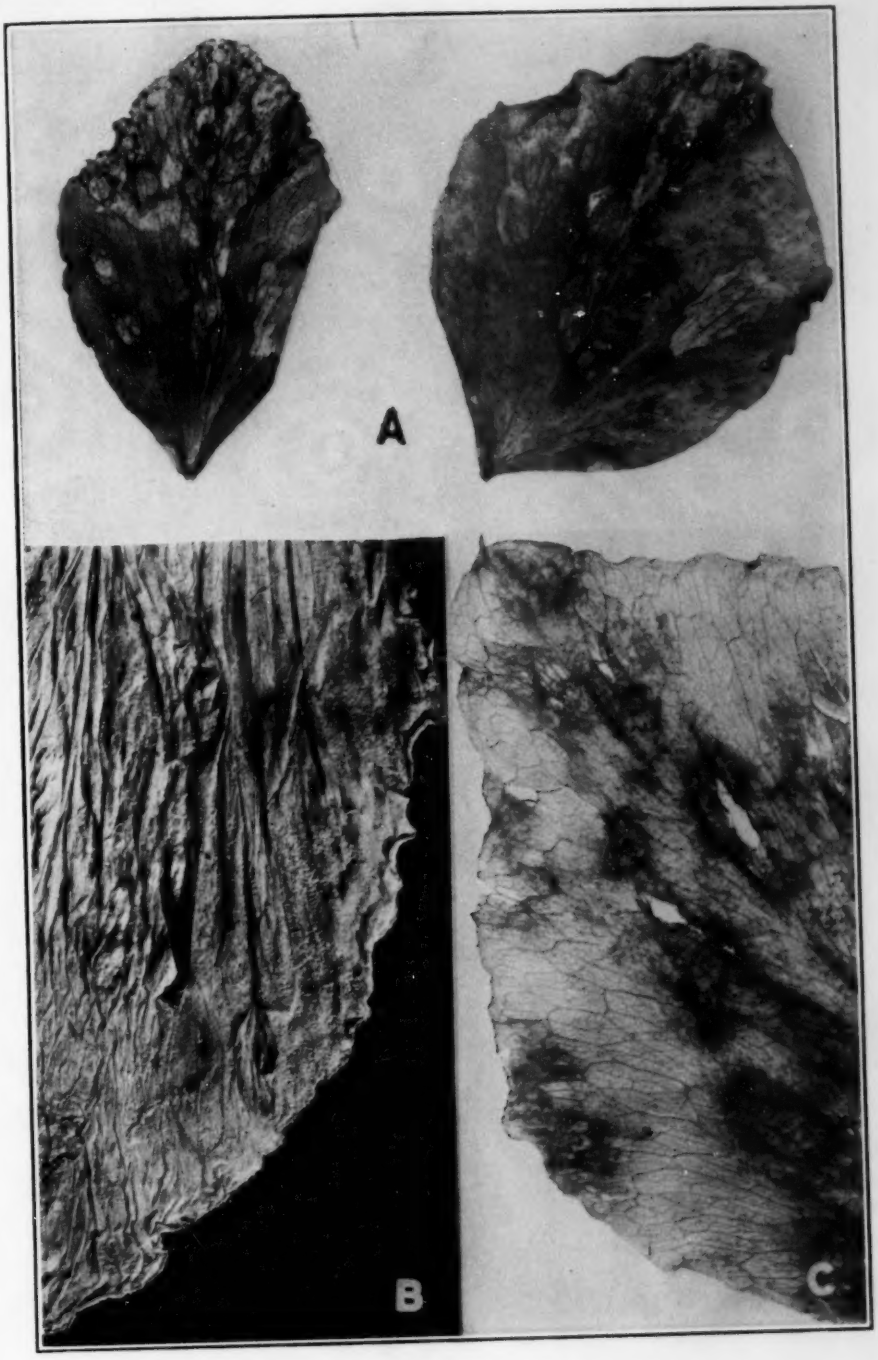
A. Germinated ascospore and formation of young perithecia. ×400.
B. Germinated ascospore and formation of *Haplaria*. ×400.
C. *Pseudofumago*. ×400.

PLATE XXVI.

A and B. Variations in *Pseudosaccharomyces* developed in culture media. ×400.

PLATE XXVII.

A. Section of a petri dish culture from spores of *Cladosporium* showing formation of perithecia. ×2.
B. Section of a petri dish culture from a single ascospore showing formation of perithecia. ×2.
C. Section of a petri dish culture showing *Haplaria*, *Pseudofumago*, and *Pseudosaccharomyces*. ×2.
D. Germinating spores and hyphal development of *Pseudosaccharomyces*. ×400.



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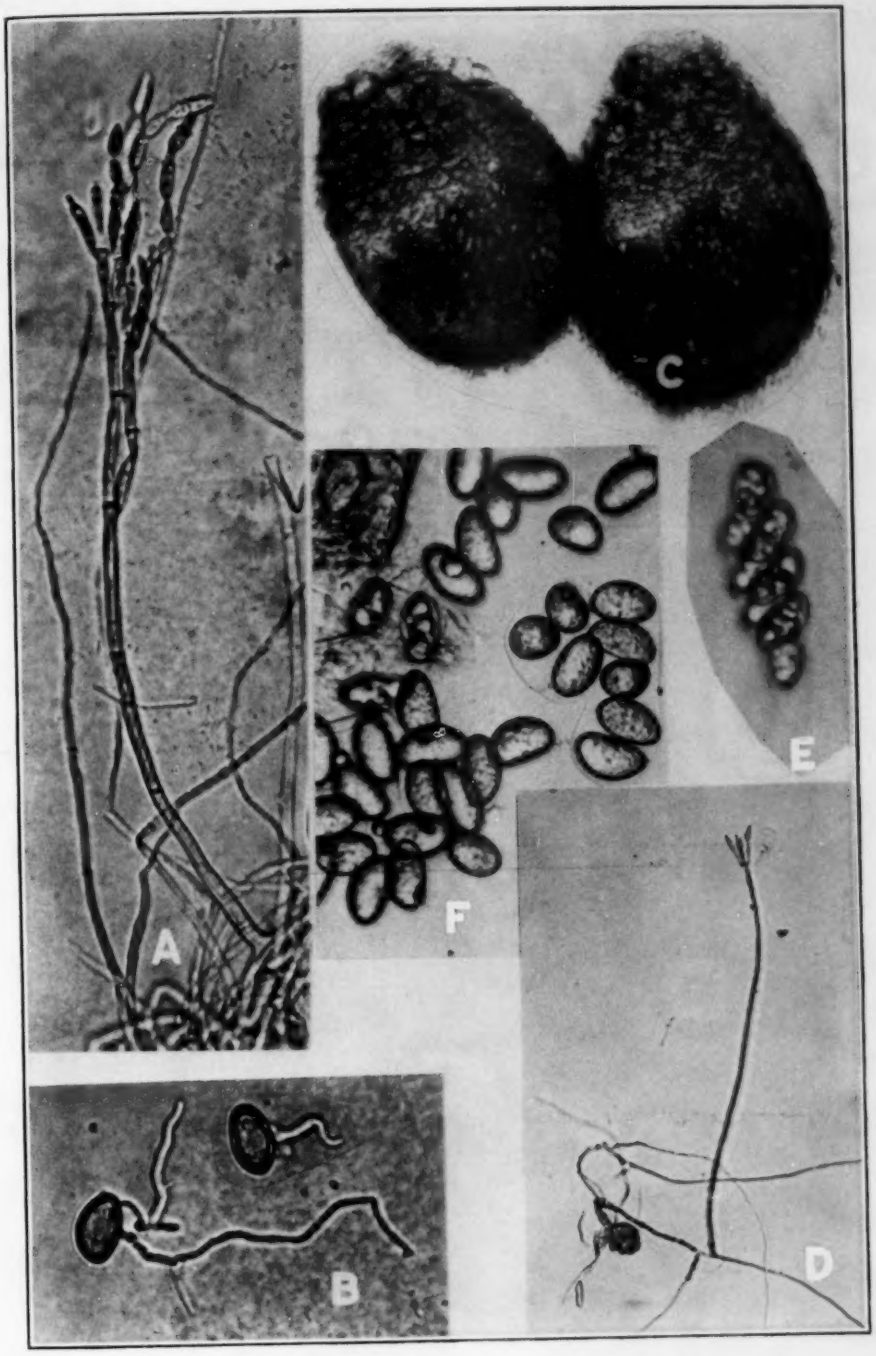

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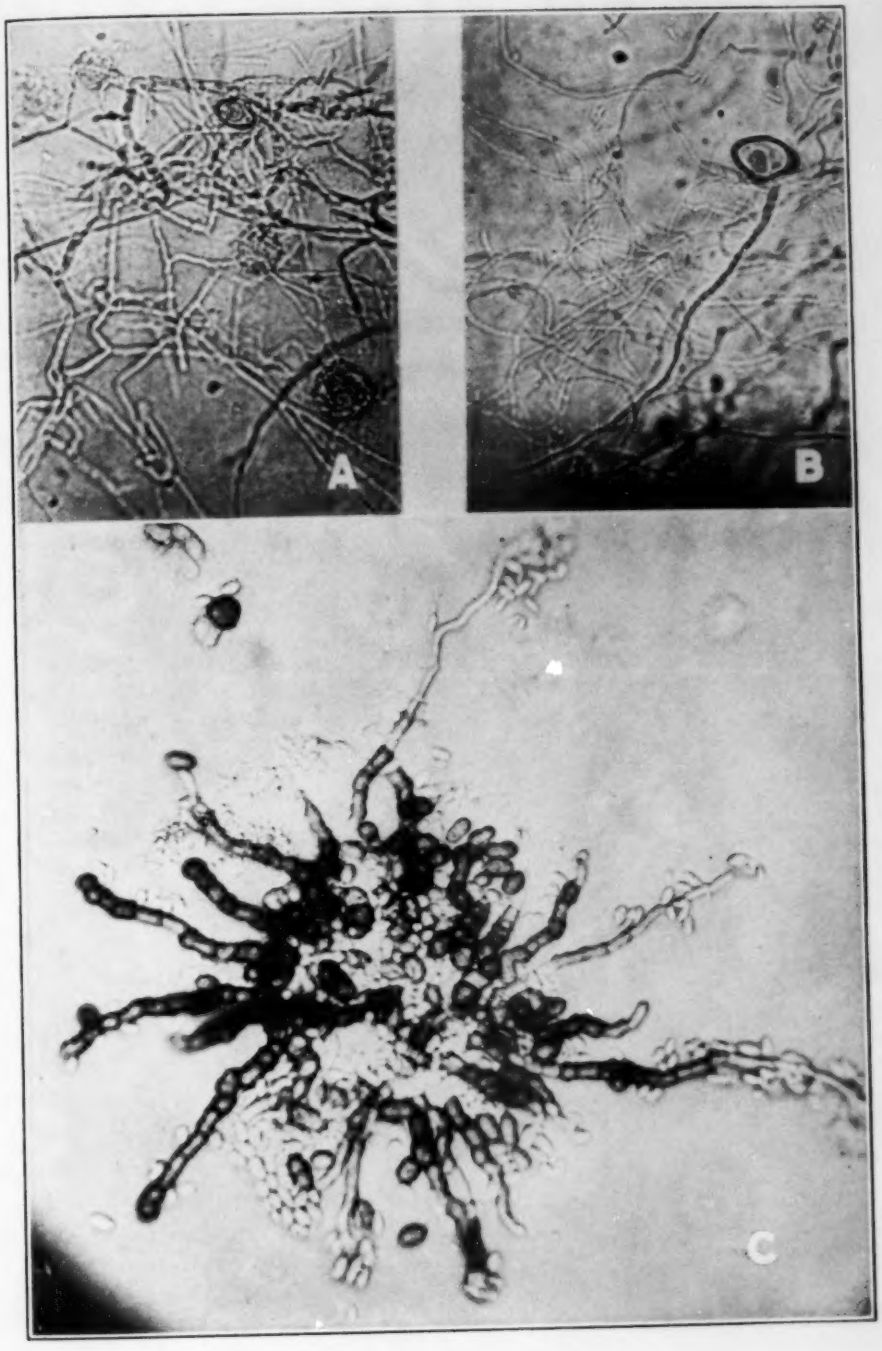

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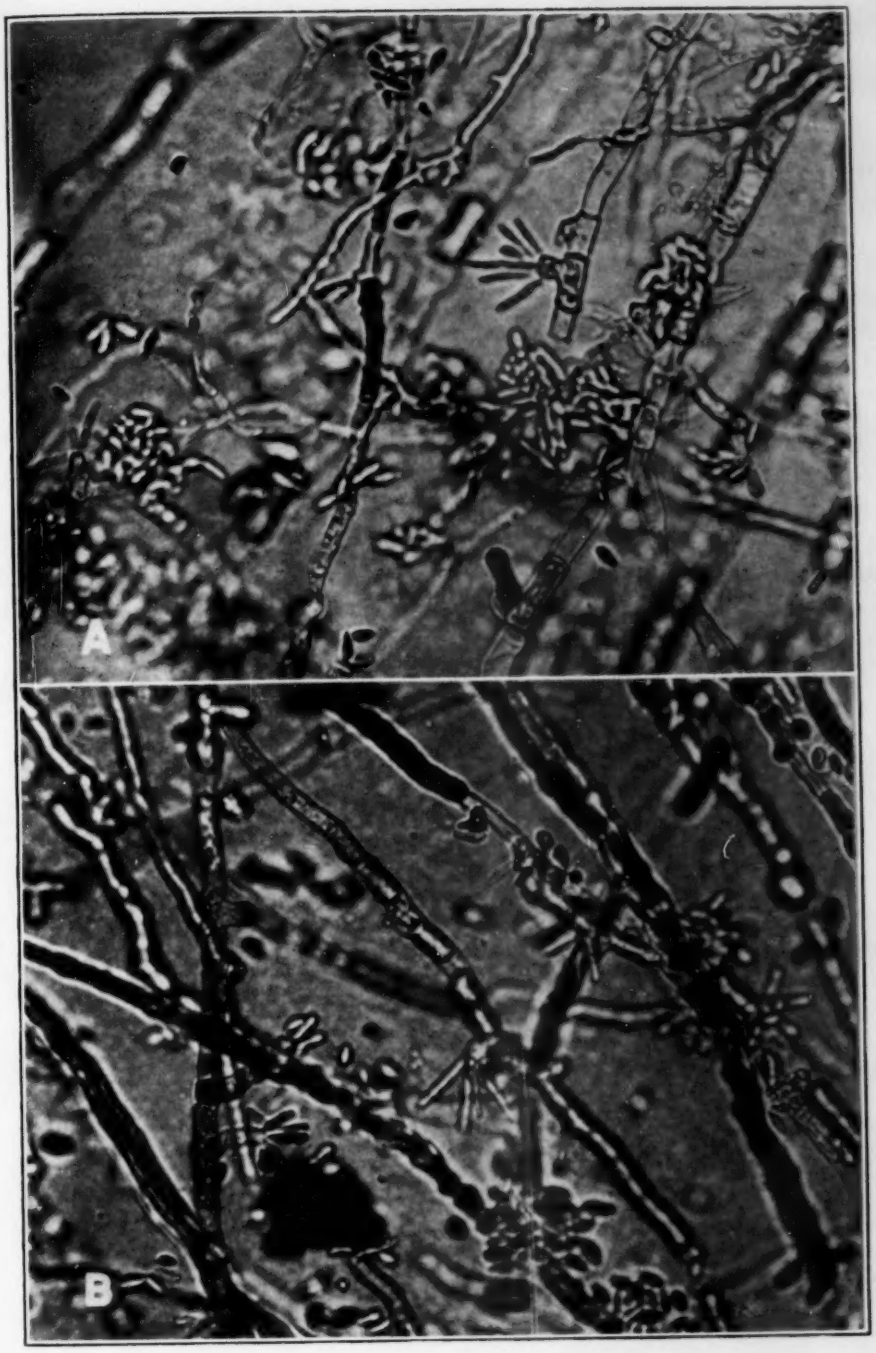

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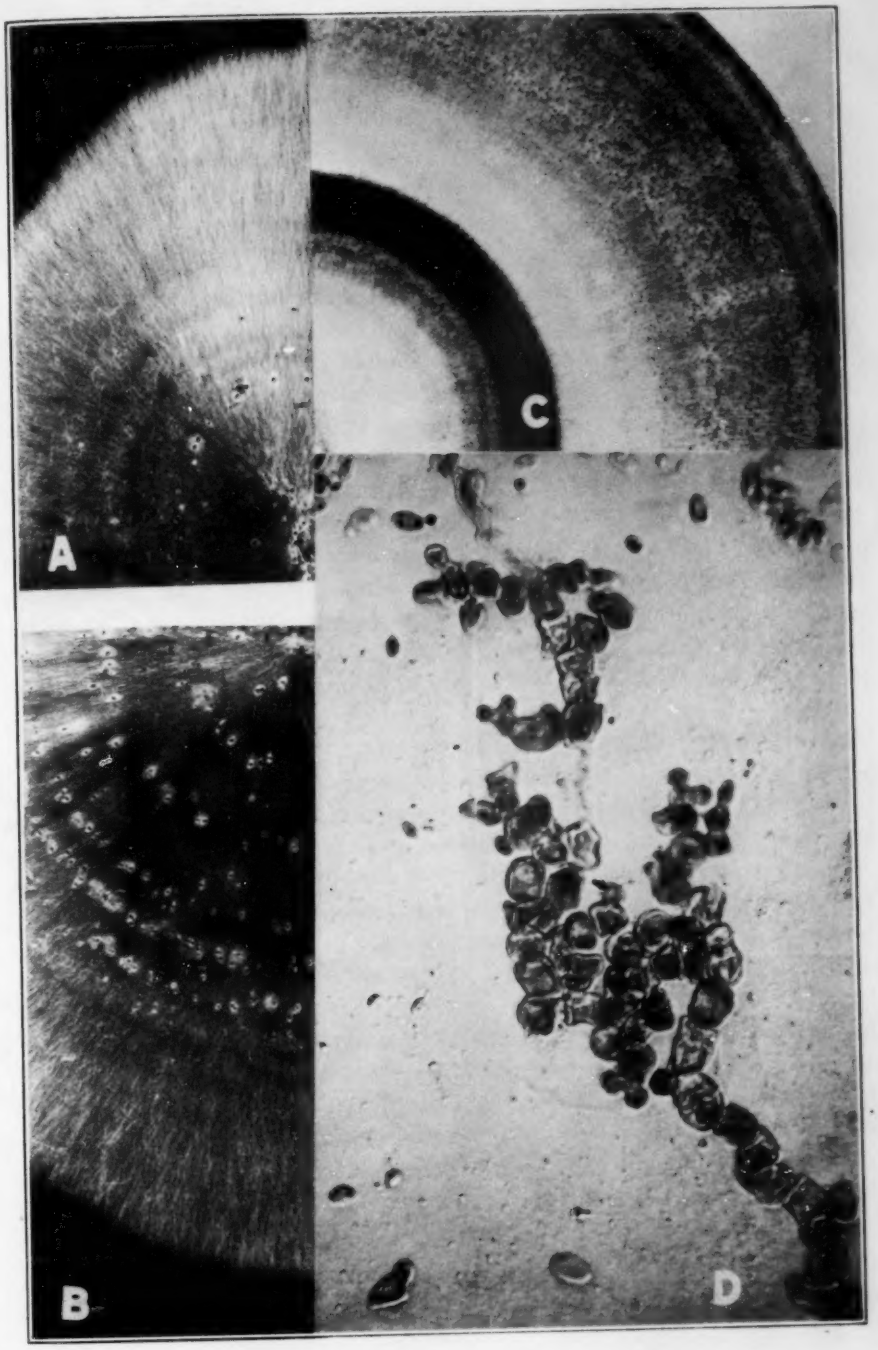

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CONTROL OF CUCUMBER POWDERY MILDEW IN GREENHOUSES[1]

E. F. GUBA

INTRODUCTION

Powdery mildew, caused by the fungus *Erysiphe cichoracearum* DC., is a common disease of greenhouse cucumbers in Massachusetts. Epidemics occur between May and October when little or no heating is practiced and during the colder months in periods of dull weather when heat is not required to maintain the minimum temperature of 60° F. for growing cucumbers. When moisture-saturated-air conditions are not prevented by heat, powdery mildew becomes epidemic. The control of this disease by regulation of the greenhouse air in the absence of pipe heat has not proved possible. The fungus yields readily to control with fungicides. Their nature, the method and rate of application under varying conditions, and their compatibility with insecticides, the use of which is also necessary, have not received previous study. Guba (3) reported the incompatibility of copper fungicides and hydrocyanic acid gas in greenhouses. This fact has prohibited the use of hydrocyanic acid gas, and tobacco fumigants have been substituted.

Both copper and sulfur fungicides are used for combating powdery mildews. Sulfur preparations are more satisfactory chiefly because sulfur offers a more lasting protection from infection and is toxic in the absence of moisture. Warm temperatures, however, are an important factor with sulfur but not with copper.

The delicate nature of greenhouse cucumber foliage and the high temperatures and bright rays of the sun to which it is subjected at times require certain precautions in connection with the choice and use of fungicides to avoid injury.

Under greenhouse conditions dusting is the most practical method of control. There are times, however, when mildew becomes prevalent on the lower surfaces of leaves where fungicidal dusts cannot be deposited. The protected position of the fungus is a handicap to its successful eradication with sulfur dusting materials during periods of cool, dull weather. Disinfection of the foliage with liquids toxic to mildew and safe for the foliage would be the logical practice to follow in such cases.

[1] Published with the appoval of the Director of the Massachusetts Agricultural Experiment Station. Contribution No. 80, 1928.

With the discovery by Whitcomb (15) of the merits of Volck, a commercial, highly refined, white lubricating oil emulsion, for controlling the Greenhouse red spider (*Tetranychus telarius* Linn.) on Greenhouse cucumbers, this oil spray has come into extensive use for this purpose. The use of Volck for red spider and of sulfur for mildew, however, is attended with injury. Since Volck is the most satisfactory insecticide known for combating red spider, and since powdery mildew and red spider frequently are present on the cucumber concurrently, there has arisen the need of fungicides the use of which would be compatible with this oil spray.

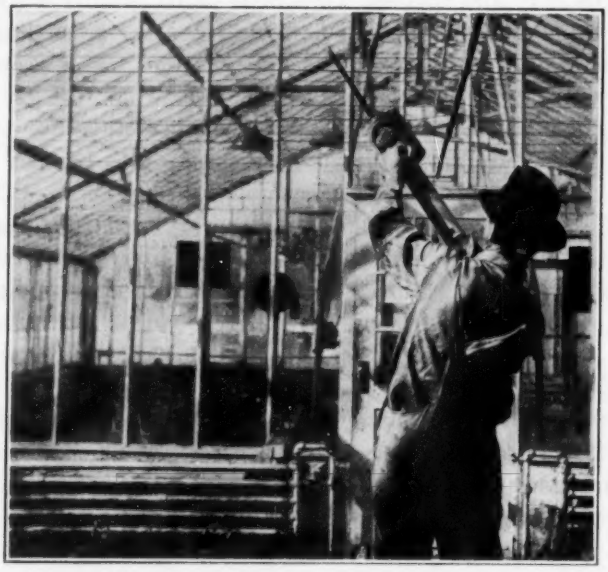

FIG. 1.—Type of hand duster most suitable for use in greenhouses showing proper method of operation for controlling cucumber powdery mildew.

CONTROL EXPERIMENTS WITH DUSTING MATERIALS

Materials Used and Methods of Application

During the period 1925–1928 inclusive, greenhouse cucumbers were dusted with various brands of sulfur. The treatments were made when powdery mildew was generally prevalent in the houses. The materials used were Grape Dust, Slug Shot, Tricked Sulfur, Sulfur-Gypsum, and Anchor Sublimed Velvet Flowers of Sulfur. Slug Shot contains 6 per cent free sulfur, Grape Dust 64 per cent, Tricked Sulfur 91 per cent, and Anchor Brand practically 100 per cent. The Sulfur-Gypsum dust was prepared to contain 15 and 20 per cent free sulfur and 80 and 85 per cent gypsum, both of 200-mesh fineness.

The Feeney Model D 2-quart duster holding 2½ pounds of dust was employed in the work. The use of this piece of equipment proved very practical in greenhouses (Fig. 1). The wires, posts, pipes, and plants offer no interference to its operation, and discharges of dust are easily obtained. Hand dusters which are strapped to the body are impractical in greenhouses.

Previous to dusting, the ventilators were closed to prevent the dust from being carried outside by air currents. Cloudy days, evenings and mornings are the best periods for dusting during the warm months of the year since at such times the ventilators can be closed long enough to permit the dust to settle without subjecting the plants to unfavorable temperatures.

The dusting of the greenhouse air was done from the walks and between the rows of vines according to the construction of the house. In houses 40 feet wide provided with walks in the center, on the sides and ends, all of the dusting was done from the walks (Fig. 2, a). In houses more than 25

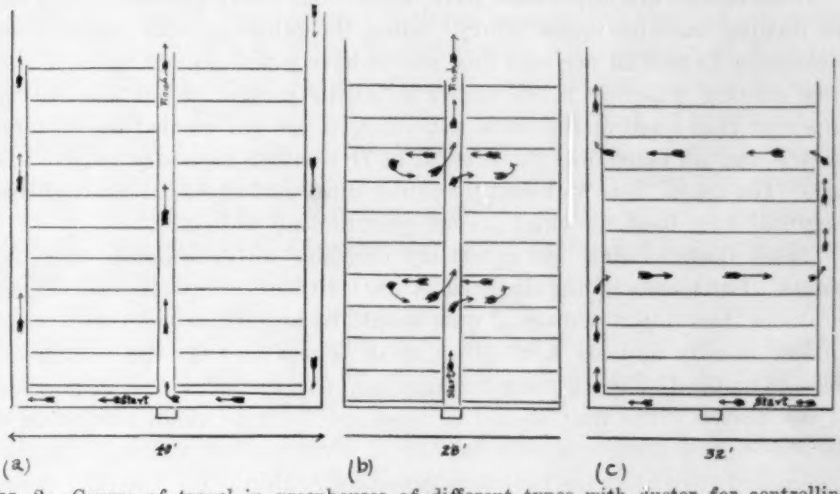

FIG. 2.—Course of travel in greenhouses of different types with duster for controlling cucumber powdery mildew.

feet wide provided only with a central walk, every 3 to 5 rows of plants were entered for about one-half the distance (Fig. 2, b). In houses with walks only around the bed, every 3 to 5 rows of plants were traversed (Fig. 2, c). At every 5 to 10 paces the discharge tube of the duster was projected above the wires overhead. One to two pushes of the plunger with the duster in an obliquely vertical position provided sufficient dust for about 500 square feet. The occasional prevalence of mildew on the lower sheltered leaves, especially where the vines were grown over the wires, re-

quired horizontal discharges of dust to destroy it. Five to six pounds of dust were used in one application in the largest houses of the dimensions 280×40 feet and where mildew was generally prevalent. Smaller quantities were used in smaller houses of where mildew was localized.

Discussion

Grape Dust, Tricked Sulfur, Sulfur-Gypsum, Anchor Brand Sulfur Dust, and Slug Shot proved excellent for dusting purposes. These materials are fluffy and fine, but Slug Shot was not so effective as the other grades. In sunlight and high temperatures characteristic of the warmer months Slug Shot has been effective in the control of mildew but not in cooler weather. The small quantity of sulfur in this material is not fungicidal under mild sunlight and temperature conditions even when in contact with mildew. Under such conditions good control has been obtained with the other sulfur dusts used.

Observations and experience have taught that heavy applications of sulfur dusting materials cause injury. Since the sulfur-gypsum preparations containing 15 and 20 per cent free sulfur have given control equal to that from dusting materials much higher in sulfur content it has also become apparent that dusting materials generally in use for combating powdery mildew contain more than the amount of free sulfur necessary to give control. The use of these high-sulfur-content dusts and excessive doses of such materials have been the chief factors contributing to injury.

Small doses of dust are extremely desirable on greenhouse cucumber plants. For houses of the dimensions 280×40 feet irrespective of volume, not more than 5 to 6 pounds of dust should be applied at one time. Since mildew usually appears first either at the doors or near the ventilators, there is no need of using the maximum quantity upon the first appearance of the disease. The dust should be used sparingly to avoid hardening of the leaves, and dusting should be started when mildew makes its appearance rather than after the fungus is generally established. The dust should be discharged into the air above the vines and permitted to settle before the ventilators are opened.

VAPORIZED SULFUR

The vaporization of sulfur has been practiced many years in greenhouses. Application of sulfur preparations to the heating pipes and the use of mechanical sulfur vaporizers (Plate XXVIII) for controlling powdery mildews have been reported by Bailey (1), Humphrey (6), Maynard (8), Höstermann (5), Norton and White (11), Norton (10), Rupprecht (12), Vogt (13, 14) and Barker and Wallace (2). Sulfuring of the heating

pipes gives control when the pipes are well heated, and is considered an effective method. Most growers have not taken kindly to the practice because the treatment corrodes the pipes, interferes with radiation, and does not always give satisfactory control. Mechanical sulfur vaporizers have lacked practical application largely because danger of losing the crop from burning sulfur has been associated with their use.

The lack of adaptation to practice of the earlier types of mechanical sulfur vaporizers led to study and use of equipment devoid of their dangerous and impractical features. The equipment that has proved most satisfactory for greenhouses consists of electric hot plates, requiring about 4.5 amperes each, porcelain evaporating dishes, asbestos board, and electric wire of No. 6 or 8 guage (Plate XXVIII e, f, g). It is operated on a 110-volt circuit and with a meter box having amperage capacity for the number of plates used. A more complete description of the apparatus and its method of operation will be presented in a paper on the control of the leaf-mold disease of greenhouse tomatoes.

The equipment was operated in large commercial rose and cucumber houses and in the range of the Market Garden Field Station at Waltham, Massachusetts. Powdery mildew has been controlled repeatedly by a single operation of the equipment, but the completeness of disinfection was associated with prevailing bright, warm weather. An extremely thin deposit of sulfur on the foliage, hardly perceptible to the naked eye, has given complete control. The strong odor of sulfur in the houses following the treatment and most pronounced on bright, warm days was a distinct feature of this method of sulfuring.

During the months of January and February, 1926, experiments were conducted on a small scale to determine the relation of the rate of application to control. Cucumber vines growing in ground beds in the greenhouse were enclosed in glass chambers of 175 cubic feet and treated with vaporized sulfur varying in rates from 1½ to 9 pounds for each 100,000 cubic feet. The temperature during the time of vaporization varied from 65° to 75° F. During the period between treatment and final observations, the temperature of the greenhouse reached 80 to 90° on five days and 75 to 80° on three other days. Equal control of epiphyllous infection was obtained by all doses, but in each case at this season of the year mildew on the sheltered foliage and lower leaf surfaces was not eradicated.

The control experiments with vaporized sulfur demonstrated the marked susceptibility of powdery mildew in contact with small doses of sulfur, and that, under continued dull or damp weather conditions, sheltered surfaces escape disinfection. The results are in line with the generally established fact that high temperatures and sunshine contribute to

the activity of sulfur at a distance. In bright warm weather vaporized sulfur has given complete disinfection of the foliage. Under such conditions the atmosphere of the greenhouse acquires a strong odor of sulfur. The action of dusting sulfur is similar but the distribution of sulfur by its vaporization is more uniform than dusting. It gives a more complete disinfection of the houses with less sulfur and the quantity of sulfur vaporized is easily regulated. After the equipment is once permanently installed its operation is convenient, but sulfur vaporization requires more time than dusting. The initial cost of equipment as well as later expense of replacing dishes and heating units is also to be considered. The extremely fine dusting sulfurs now available and the ease with which they are applied hardly justify the vaporization of sulfur on the hot pipes or with mechanical apparatus; neither would the practice be so profitable a means of control for commercial establishments as dusting. If vaporized sulfur were the most effective and practical means of obtaining control, as has been demonstrated for tomato leaf mold, caused by *Cladosporium fulvum* Cke., the practice would be warranted.

CONTROL EXPERIMENTS WITH LIQUID FUNGICIDES

Under dull or damp weather conditions the spread of powdery mildew on lower and sheltered leaf surfaces cannot be suppressed with sulfur dusting materials. Serious attacks of powdery mildew confined to lower leaf surfaces have been noted in commercial greenhouses which could not be controlled with sulfur dust. Experiments were conducted to determine the merits of liquid fungicides under such conditions.

Volck as a Fungicide

McWhorter (9) reported control of rose mildew, *Sphaerotheca pannosa* (Wallr.) Lév., with Volck. As far as the writer is aware this is the only published report of the fungicidal efficiency of this oil spray. Three treatments of Volck (1 pt. to 4 gals. of water) were applied at intervals of two to three weeks. The season's control of rose mildew from these treatments was considered remarkable.

The writer used Volck in a series of tests in the greenhouse on mildewed cucumber foliage. The 1 per cent strength recommended for controlling red spider was used. At this strength, the oil spray occasionally was somewhat toxic to mildew. Instances have been noted of toxicity only in the centers of mildewed areas, while the margins remained perfectly healthy. Usually treatment with the oil spray was followed within 24–48 hours after application by the appearance of numerous new infections, which indicated that the spray was directly responsible for this spread. In some

instances total elimination of the fungus was observed but there was no protection from subsequent infection. Lack of control was outstanding. The value of Volck in respect to its fungicidal activity to powdery mildew of cucumbers is not significant. Its use alone for red spider when powdery mildew is also present cannot be considered as fulfilling the requirements for control of the latter.

Copper Sprays

Bordeaux mixture is fungicidal to powdery mildew. Tests with 1–1–100, 2–2–100, and 4–4–100 mixtures were made. The first two mixtures left no objectionable residue on the fruit and foliage and the third left a heavy residue and a hardened foliage. All mixtures have consistently given equal control. Certain dry and liquid copper fungicides diluted to contain the same amount of metallic copper in 1–1–100 Bordeaux spray, *i.e.*, 0.03 per cent, proved equally active against mildew. Those considered were Basic Copper Sulfate prepared by Holland *et al.* (4) containing 26 per cent copper, Hammond's Copper Solution 3.05 per cent, Dow Powdered Bordow 12.5 per cent, and Grasselli Bordeaux Mixture Powder 13 per cent.

Copper sprays are necessary when atmospheric conditions render sulfur dusting materials ineffective, but contact of the spray with mildew is necessary to obtain eradication. The use of fine, up-turned nozzles and high pressures are essential to insure wetting of the lower sides of the leaves.

Copper fungicides do not offer protection from infection. This fact was clearly demonstrated in an experiment in which cucumber vines were sprayed on August 26 with bordeaux mixtures, 1–1–100 and 2–2–100, with and without saponin added to assist wetting. Equal control was obtained in each case, but mildew appeared again, necessitating the use of sulfur on September 6, eleven days after the copper application. Similarly, 20–80 monohydrated copper sulfate-lime dust is not toxic to mildew on dry foliage. The same dust gives control if the foliage is syringed with water immediately before or after dusting. Lack of protection from infection with copper spray residues is due to the fact that the fungus develops on the foliage in the absence of moisture, which is essential to copper activity. Copper sprays, however, are extremely useful during cool, dull weather when sulfur offers no control.

Sulfur Sprays

Commercial liquid lime-sulfur concentrate, potassium sulfide, and dry mix sulfur lime were considered. The tests were made under bright weather

conditions in September, 1927, and April, 1928, at temperatures above 85° and 90° F. Lime-sulfur concentrate was used at dilutions ranging from 1–200 to 1–1000. Below the dilution of 1–200, burning and a conspicuous residue resulted and the foliage acquired a hardened texture. Dilutions from 1–200 to 1–1000 were fungicidal, but the stronger of these left an objectionable residue on the cucumber. Potassium sulfide at stronger than 1–400 dilutions caused burning. This fungicide at 1–400 to 1–1000 dilutions was fungicidal to mildew, caused no injury and left no perceptible residue. Dry mix sulfur lime sprays of 2, 3 and 4 per cent suspensions proved safe but their residues on the fruit render this material undesirable.

Potassium sulfide, 1 pound to 50 gallons of water, may be used in place of bordeaux 1–1–50 for combating lower leaf surface infection. It gives slightly better protection than bordeaux, altho the choice of either type of spray should be influenced by its compatibility with insecticides recommended for controlling insects and red spider.

COMBINATION TREATMENTS

Copper Sprays and Volck

The absence of control with Volck and the possibility of increasing the prevalence of powdery mildew rather than suppressing it led to consideration of a combination of the oil spray with copper fungicides. The advantage of a combination spray which would control red spider and powdery mildew with one treatment was obvious.

It was found that acid solutions and Volck were incompatible. The former separated the oil from the emulsion. For this reason weak dilutions of copper sulphate and sulfuric acid could not be considered. All copper fungicides considered gave alkaline reactions in water and produced compatible mixtures with this oil spray. These were Holland's Basic Copper Sulfate, Hammond's Copper Solution, Dow Powdered Bordow, Grasselli Bordeaux Mixture Powder, and freshly made bordeaux mixture 1–1–100. Temperatures of 84–86° F. prevailed at the time of application with lower temperatures not below 50° F. during evaporation of the spray. All of these combinations with Volck gave equal control but Volck alone gave little or none.

In another experiment, plats of cucumber vines were treated on August 1 with Volck 1 per cent, Volck 1 per cent and bordeaux 1–1–100 combined, and bordeaux 1–1–100. The foliage sprayed with the oil emulsion alone showed, a few days after application, numerous new centers of infection, and mildew had by August 8 assumed such prevalence that bordeaux 1–1–100 was applied to suppress it. The application of bordeaux on August 1 gave excellent

control and the foliage remained free of mildew until August 12, but further treatment was not considered necessary until August 18 (Fig. 3). Volck and bordeaux 1–1–100 combined and bordeaux 1–1–100 alone proved equally effective as disinfectants. The combination spray spread much better than

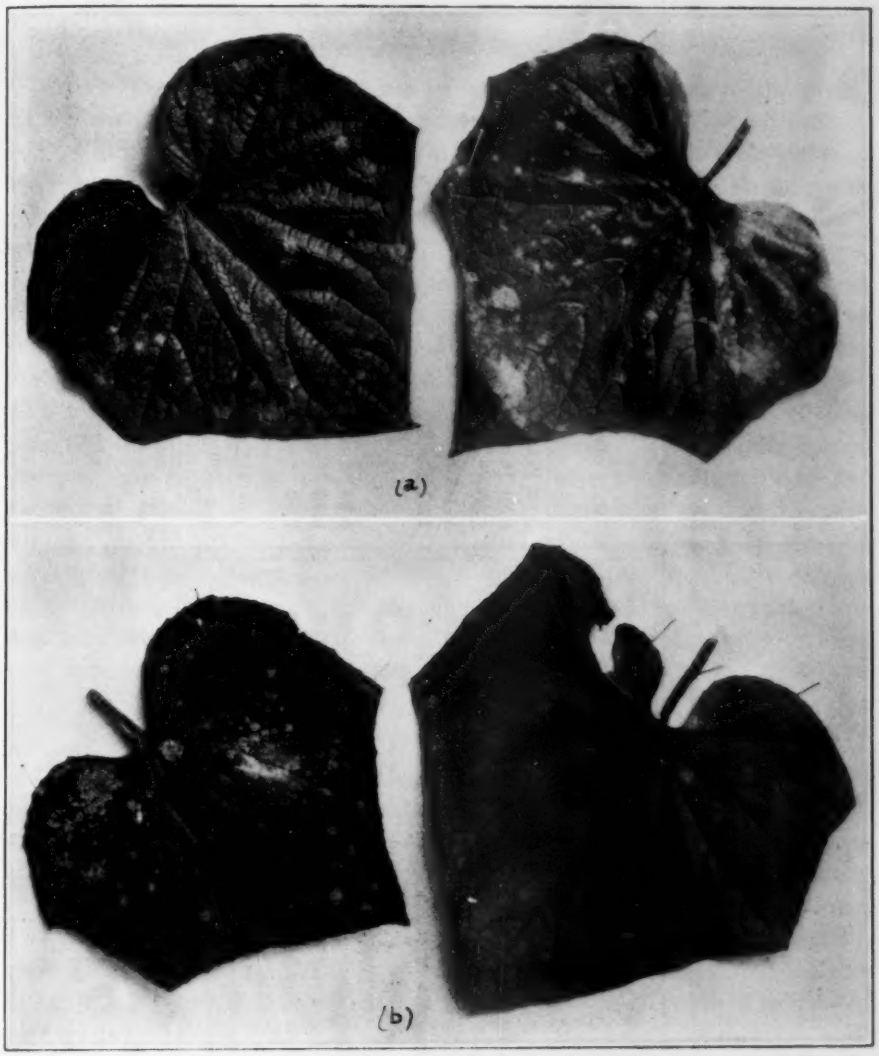

FIG. 3.—Cucumber leaves. (a) At left treated with bordeaux 1–1–100 containing 1 gallon of Volck concentrate. At right treated with Volck of the same strength. (b) At right sprayed with bordeaux 1–1–100, at left no treatment. Photographs six days after application.

TABLE 1.—*Toxicity of copper fungicides to powdery mildew and injury to foliage following fumigation with hydrocyanic acid gas*

Materials	Copper content of concentrate (per cent)	Rate of dilution	Toxicity to foliage	Toxicity to mildew	Injury with hydrocyanic acid gas
Basic Copper Sulfate	26.00	0.96 lb.–100 gals.	–	+	Moderate
Hammond's Copper Solution	3.05	8.19 " –100 "	–	+	Severe
Dow Powdered Bordow	12.50	2.00 " –100 "	–	+	do
Home-made bordeaux	25.00	1–1–100	–	+	do
Grasselli Bordeaux Mixture Powder	13.00	1.91 lb.–100 "	–	+	do
Water			–	–	None
Volck Spray 1 per cent			–	–	do
Volck and Basic Copper Sulfate	26.00	0.96 " –100 "	–	+	Slight
Volck and Hammond's Copper Solution	3.05	8.19 " –100 "	–	+	Moderate
Volck and Dow Powdered Bordow	12.50	2.00 " –100 "	–	+	Slight
Volck and Grasselli Bordeaux Mixture Powder	13.00	1.91 " –100 "	–	+	Severe
Volck and home-made bordeaux	25.00	1–1–100	–	+	Slight

Volck. Since complete wetting of infected foliage is essential to total disinfection, this improvement in the wetting of the oil spray resulting from the addition of bordeaux mixture was considered a distinct advantage.

Copper Sprays and Hydrocyanic Acid Gas

According to Guba (3) the use of hydrocyanic acid gas in the greenhouse when copper deposits are present on foliage is attended with severe foliage burning. Foliage sprayed with bordeaux mixture 1–1–100 and later exposed to hydrocyanic acid gas was severely burned. Injury also occurred from using 0.04 per cent copper sulphate solution on tomato plants when subjected to the gas.

Rows of cucumber plants were sprayed with different brands of copper fungicides alone and in combination with Volck 1 per cent, and diluted on the basis of copper contained in 1–1–100 bordeaux spray, *i.e.*, 0.03 per cent metallic copper. One week after application the greenhouse was fumigated with hydrocyanic acid gas generated from calcium cyanide. The latter was used at the rate of ¼ ounce to each 1,000 cubic feet. When copper sprays were used in combination with the oil spray, injury was slight with two exceptions (Table 1). When copper sprays alone were used injury was severe and of about the same degree. The oil emulsion apparently provides some protection to the copper residue from being acted upon by the fumigant. Injury is serious enough to make inadvisable the use of hydrocyanic acid gas when the combination is used. Tobacco fumigants or sprays should be substituted in such cases.

Sulfur Fungicides and Volck

Sulfur fungicides and Volck are not compatible. In the majority of cases called to the writer's attention injury occurred from using Volck after sulfuring. Injury was also observed in one establishment where the oil was used three times on the same crop in the absence of sulfur. Injury is manifested in the form of yellowing of the leaf accompanied by burning. The foliage appears oily and glossy and when dried is semi-transparent, resembling parchment. Finally the leaves turn brown and crisp. Manifestation of injury is rather slow and apparently related to the slow rate of penetration of the oil.

Young cucumber plants were treated with Volck and Grape Dust, Tricked Sulfur, and Slug Shot. Injury occurred in either order of application of oil emulsion with Grape Dust and Tricked Sulfur, but none with Slug Shot. Plants treated with these dusting materials or with oil emul-

sion alone were not injured. In further experiments sulfur dust containing 15 and 20 per cent free sulfur have proved compatible with this oil spray. Inferentially the compatibility of Slug Shot with Volck is due to its small sulfur content. Since 15 and 20 per cent sulfur dusts are fungicidal to the fungus, sulfur dusts of higher contents are not necessary. High grade sulfur dusts should not be risked on cucumber vines that have been, or are to be, sprayed with Volck. If the use of the oil spray for red spider is an established necessity, low grade sulfur dusts should be used to combat mildew. Dusting materials containing 15 and 20 per cent free sulfur are of much greater merit than Slug Shot and can be prepared at a great saving in cost.

In further experiments sulfur spray materials were compared for their compatibility with Volck. Half rows of plants were sprayed with 0.5 and 1 per cent lime-sulfur, 0.13 per cent potassium sulfide, and 5 and 12 per cent dry-mix sulfur lime. Three days later the entire rows were sprayed with 1 per cent Volck. Lime-sulfur 1 per cent caused burning, but the other sulfur sprays none. Following the oil emulsion treatment the foliage of the sulfured vines gradually turned yellow. Injury was most severe in the plats receiving 1 per cent lime-sulfur and dry-mix sulfur lime. The foliage on the other half of each row, which was sprayed with oil emulsion only, remained healthy. Nine days after the oil emulsion application these half-rows which received only the oil spray were sprayed with sulfur fungicides corresponding to treatments given the other half of the rows. Severe burning appeared in the plat treated with 1 per cent lime-sulfur and more or less yellowing and burning resulted from the other treatments, except from potassium sulfide 0.13 per cent. Injury was more pronounced when the oil spray followed sulfur than where the order of application was reversed. This is in line with experiences growers have had in using sulfur and Volck.

During bright weather of April, 1928, and prevailing high greenhouse temperatures vines were sprayed with a 2 per cent suspension of dry-mix sulfur lime, 0.25 per cent potassium sulfide, 0.5 per cent lime-sulfur, and 1 per cent Volck. Two vines in each plat received the 1 per cent Volck treatment in addition to the sulfur sprays. Vines receiving both the oil emulsion and sulfur treatments showed yellow foliage and burning which gradually became very serious. The results have shown that sulfur sprays can not be used on greenhouse cucumbers which require the oil spray for combating red spider.

SUMMARY

Powdery mildew on cucumbers in greenhouses is readily controlled with fungicides, but their choice requires caution to avoid dangerous combina-

tions with insecticides, and their strength and rate of application require modification to prevent injury.

Sulfur fungicides are most effective for combating cucumber powdery mildew in greenhouses. Slug Shot, containing 6 per cent free sulfur, is fungicidal only in bright weather and high temperatures. Sulfur dusting materials containing 15 and 20 per cent free sulfur and high grade dusts containing 64, 91, and 100 per cent free sulfur have given equal control. The latter should be used sparingly to avoid injury. Low grade sulfur dusts are safer than high grade sulfur dusts, and therefore are preferable.

Applications of sulfur dust should not exceed 5–6 pounds in houses 280×40 ft. Smaller quantities should be used in smaller houses or when mildew is localized.

In cool or damp weather sulfur dusting materials are not effective and do not control lower leaf surface infection. Under such conditions copper or sulfur sprays have proved effective.

Bordeaux 1–1–50 is preferable to stronger mixtures. The latter harden the foliage and leave residue on the fruit. Proprietary copper fungicides containing an equivalent amount of copper are fungicidal. Disinfection is obtained by wetting.

Commercial lime-sulfur concentrate is safe in dilutions from 1–200 to 1–1000 and potassium sulfide in 1–400 to 1–1000. Stronger dilutions are toxic to the foliage. Lime-sulfur 1–200 and potassium sulfide 1–400 provide slightly better protection than copper sprays. Potassium sulfide does not leave a noticeable residue, and therefore is preferable to lime-sulfur.

Highly refined, white lubricating oil emulsion (Volck) exhibits mild fungicidal activity to mildew but does not control it.

Sulfur fungicides and Volck are not compatible. High grade sulfur dusts, 0.5 per cent lime-sulfur, 0.25 per cent potassium sulfide and 2 per cent dry-mix sulfur lime sprays caused injury when used before or after the oil spray. Slug Shot and low grade sulfur dusts containing 15 and 20 per cent free sulfur are compatible.

Volck may be combined with bordeaux or with proprietary copper fungicides. Bordeaux 1–1–50 or proprietary materials diluted to contain an equivalent amount of copper are recommended. The mixture is recommended if mildew is present when the oil spray is required for red spider.

Copper fungicides and hydrocyanic acid gas are incompatible. Fumigation with hydrocyanic acid gas should not be practiced if copper residues are present on the foliage. Tobacco sprays or fumigants should be used in such cases.

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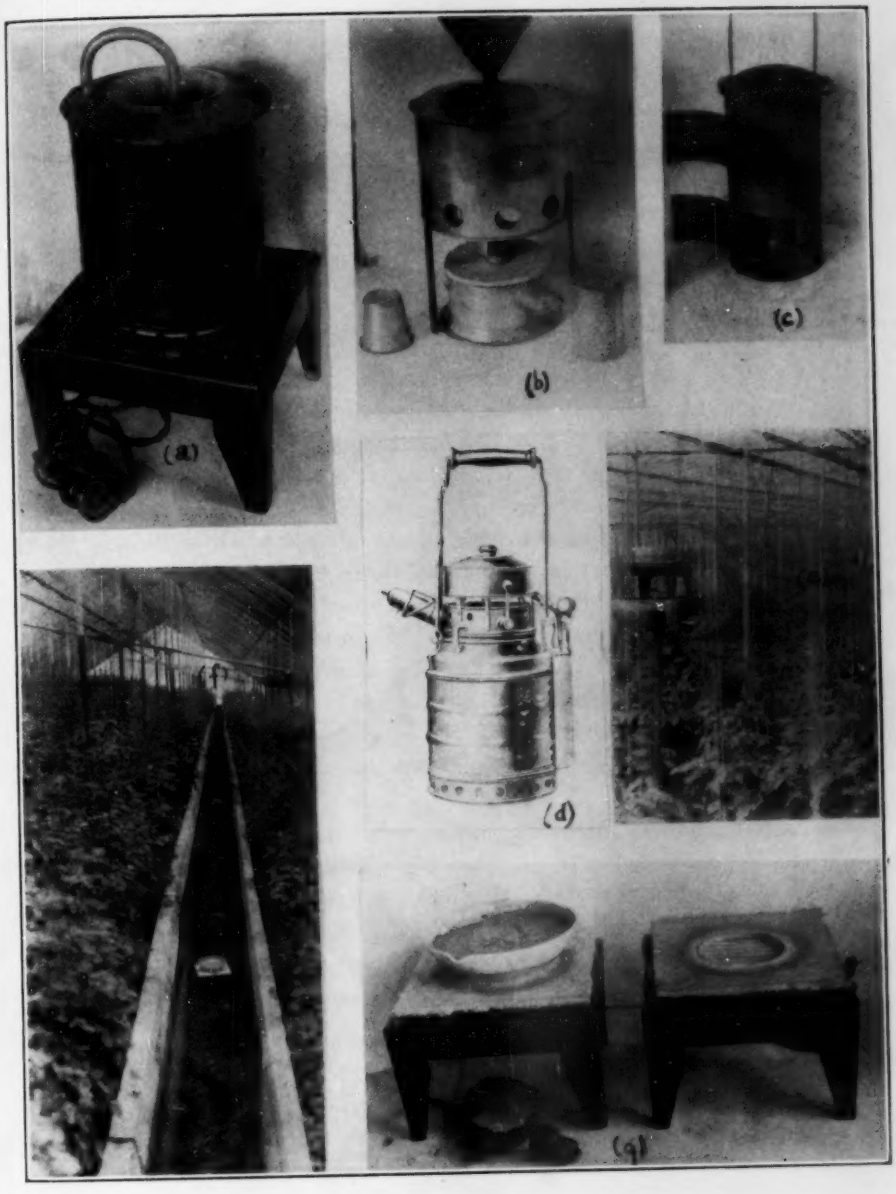
EXPLANATION OF PLATE XXVIII.

(a), Sulfur Sublimer; (b), Campbell's Patent Sulfur Vaporizor; (c) Dorrance Sulfur Burner; (d), Rota-Generator; (e), Vaporizing equipment assembled in tomato house; (f), Vaporizing equipment assembled in rose house; (g), Electric hot plate, asbestos board, and porcelain evaporating dish assembled for operation.


(a)
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THE ROOT-KNOT OF ABACÁ, OR MANILA HEMP[1]

GERARDO OFFIMARIA OCFEMIA AND MELANIO R. CALINISAN

THE DISEASE

Root-knot is a very common, though not usually serious, disease of abacá, or Manila hemp (*Musa textilis* Née) in the Philippine Islands. In severe cases and in advanced stages of the disease the roots of the plants turn brown, die, and rot. On account of the death of all, or of the greater part of, the roots, infected abacá plants become much stunted, their leaves become small and pale green or yellowish, and the petioles may be more or less bunched up at the upper end of the pseudo-stem. Owing perhaps to the common occurrence of root-knot on abacá in Cavite and Laguna provinces, where the bunchy-top disease is most destructive, Hernandez (5), Teodoro (10) and Teodoro and Serrano (11 and 12), all of the Philippine Bureau of Agriculture, believe that bunchy-top is caused in part, at least, by *Heterodera radicicola*. Fahmy (3) in Egypt describes a disease of banana, a relative of the Manila hemp, which is due to a *Heterodera* different from *H. radicicola*. This author states that the disease causes dwarfing of the banana and the production of small upright leaves which are arranged in the form of a rosette. Fahmy (3) reports that when more than 50 per cent of the roots of the banana are affected the rosette condition is distinct and many of the roots are rotted. The disease is also said to cause the pseudo-stem to split, and later the inner tissues rot. According to Magee (6) the Egyptian banana trouble is identical with the bunchy-top disease in Australia.

The present paper reports the results obtained in experiments conducted from 1925 to date which show that the nematode occurring in the galls of abacá roots and in the soil in fields where the bunchy-top disease occurs, causes a malady entirely different from the bunchy-top (7 and 8).

Symptoms

On aërial parts.—The indication on the aërial parts of the plant that abacá is infected by root-knot may be noted on the leaves. They become

[1] Mr. Calinisan used part of the data herein reported in a thesis presented by him for graduation with the degree of Bachelor of Agriculture from the College of Agriculture, University of the Philippines, March, 1928. He was responsible for experiments carried on from April, 1927, to January, 1928, and for the study of the nematode.

Contribution from the Experiment Station of the College of Agriculture at Los Baños, Laguna, Philippine Islands. Published with the approval of the Director.

pale green to yellowish and this paleness is more pronounced in the youngest leaf. At first not much difference may be seen between healthy (Fig. 1, A) and infected plants (Fig. 1, B) except in size. As the disease advances, however, the leaves gradually become shorter and narrower than those of healthy plants of the same age. The plant becomes stunted and the leaves tend to crowd at the upper end of the pseudo-stem. The symptoms exhibited by the aërial parts of the abacá, however, are not reliable because other conditions, such as poverty of the soil, or lack of available moisture in it, crowded planting of abacá seedlings, and mechanical injuries to the roots may cause exactly the same changes.

On the underground portions.—The most reliable symptom of root-knot is the presence of the galls in the roots (Fig. 2, B). The galls are of varying shapes and sizes, depending upon their location on the roots. They may be from 3 to 10 or more mm. in diameter. Several of these galls may run together so that the infected portion of the roots may appear as an irregular, almost club-shaped body, sometimes 5 cm. long by 1 or more

FIG. 1. Abacá seedlings, 3 months and 22 days old, of variety Sinibuyas showing the relative size of plants grown in sterilized nematode-infected soil (A) and unsterilized nematode-infected soil (B) after two months and 17 days. Note that the plants in B, though much stunted, do not show symptoms of bunchy-top. (Photograph by the photographic division, department of agronomy, College of Agriculture, Los Baños, P. I.)

cm. in thickness. At first the roots are normal in color, or almost yellowish white, but later they become brown to almost black. The surface of the galls becomes rough with age on account of the cracking of the epidermis and cortex. In advanced stages the roots die so rapidly that new ones are developed, but these are finally infected and killed.

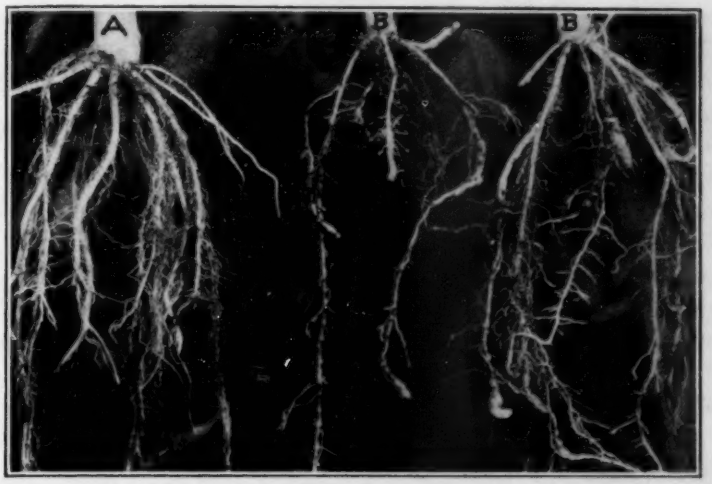

FIG. 2. Three root systems of the abacá seedlings shown in figure 1, A and B. At the left (A) is the root system of one of the plants in the sterilized infected soil after allowing the plants to grow in it for two months and 17 days. The middle and right root systems (B) are those of plants in the unsterilized infected soil showing almost club-shaped roots due to galls produced by *Heterodera radicicola*. (Photograph by the photographic division, department of agronomy, College of Agriculture, Los Baños, P. I.)

CAUSAL ORGANISM

Egg.—The egg of *Heterodera radicicola* is almost elliptical, or cylindrical with rounded ends. According to Byars (2) the eggs are about 88×35 μ. Godfrey (4) reports that usually the egg of *H. radicicola* is less than 1/250 inch, or about 100 μ in length. Measurements made at Los Baños of 50 eggs of the abacá root-gall nematode showed that the size range is 76–108 × 36–48 μ. The average size of the eggs is 91.8×41.3 μ, or 3.8 μ longer and 6.3 μ thicker than those reported by Byars (2). The embryo ranges from 80–112 × 36–48 μ. The average size is 93.9×41.1 μ.

Larva.—Godfrey (4) states that the length of a newly hatched larva of *H. radicicola* is about 1/80 to 1/50 of an inch, or about 312.5 to 500 μ, and the diameter of the body is about 1/30 of its length or about 10.4 to 16.6μ. According to Byars (2) the larvae are about 400 μ in length. Fifty larvae

of the male abacá-root nematode were measured and the size range of these was 331.6–464.2 × 16.5–25.9 μ. The average size is 409.5 × 18.8 μ. Compared with Godfrey's (4) figures the larvae of the abacá nematode have an average length which lies within the range given by him and an average thickness which is 2.2 μ greater. The larva molts several times, but no work was done to determine the number of molts before maturity is reached.

Measurements of 50 female larvae show that the size range is 352–547.1 × 32–91.1 μ. The average size is 432.3 × 50.7 μ. The size range of the female nematode approaching maturity is 416.7–769.4 × 144.3–432.8 μ. The average size is 514.7 × 269.1 μ.

Male.—The male abacá nematode is long, of a typical cylindrical shape, and tapering at both ends. Baylis and Daubney (1) state that the body of the worm is covered with cuticle which is striated, but it has no bristles. The head of the male is distinct, and there are six lips. The stylet is composed of three rods, which are fused throughout and knobbed behind. It has two testicles. The posterior end has no alae or papillae. The spicules are equal, short, and broad. Godfrey (4) found that the mature male nematode measures from 1/20 to 1/16 of an inch in length, or about 1,250 to 1,562.5 μ, but not over 1/40 as thick as it is long, or about 31.25 to 39.06 μ. Byars (2) reports that the mature male is a little more than 1 mm. long. Scofield (9) states that the size of the male nematode is from 1 to 1.5 mm. long.

Female.—The body of a mature female abacá nematode becomes swollen into an ovoid or pear-shaped structure. Only the neck portion of the worm remains normal. According to Baylis and Daubney (1), in the female nematode there is a terminal prominence which takes the place of the tail and this carries the vulva. The anus becomes located on the dorsal side. Baylis and Daubney (1) further state that *Heterodera radicicola* is oviparous. The eggs, however, remain in the uterus. After the death of the female the eggs hatch and the larvae then escape.

In regard to size Byars (2) states that the greatest diameter of the mature female *Heterodera radicicola* is about 0.5 mm., or 500 μ. Godfrey (4) reports that the diameter of the female nematode is from 1/40 to 1/25 of an inch, or about from 625 to 1000 μ. According to Scofield (9) the mature female nematode is less than 1 mm., but oftentimes it is about 3/4 of a millimeter, or 750 μ in length. The writers found that the size range of the mature female nematode of the abacá is 720–1280 × 400–800 μ. The average size is 1056.1 × 619.5 μ.

The size of the eggs of the abacá nematode in the Philippines is nearly the same as that of Byars (2) and Godfrey (4) for the eggs of *H. radicicola*. The characteristics of the male and female nematode agree with

those given by Baylis and Daubney (1) for *Heterodera*. The presence of two testicles in the mature male shows that it is *H. radicicola*. In order to confirm the writers' determination, specimens were submitted to Marcos A. Tubangui of the Department of Veterinary Parasitology, College of Veterinary Science, Los Baños, Laguna; he identified them as *H. radicicola*.

THE RELATION OF HETERODERA RADICICOLA TO ABACÁ, OR MANILA HEMP AND TO THE BUNCHY-TOP DISEASE

It was first proved by the senior author (8) in August, 1925, that the bunchy-top of abacá in the Philippines is caused by virus which is transmitted from diseased plants to healthy abacá by the aphid *Pentalonia nigronervosa* Coq. Experiments were conducted to determine if bunchy-top could be transmitted by planting healthy abacá seedlings in soil which was collected from areas infected with bunchy-top in Laguna and Cavite provinces. The soil in the bunchy-top-infected areas of Laguna and Cavite and the roots of the infected abacá contain numerous nematodes. On account of the constant association of nematodes with roots of abacá infected with bunchy-top, these eelworms have been suspected of being the cause of bunchy-top (5, 10, 11, and 12). In the experiments which are reported in this paper, 24-centimeter pots, or 5-gallon kerosene cans which were cut into halves, were filled with the nematode-infected soil taken from Laguna and Cavite. One of the pots or cans of soil was always sterilized for a check in the autoclave for two hours at 15-pounds' pressure. The pots were planted with three to five seedlings of abacá which were grown from seeds. After planting the seedlings the pots were placed on a wooden bench out of doors where they were watered at rgular intervals.

After from 38 to 51 days it was noted that the checks were much taller and darker colored than the plants in the unsterilized soil (Fig. 1, A and B). All of the plants were then dug up and their roots were carefully examined. It was always noted that galls of various sizes were present on the roots of all plants grown in the unsterilized soil, while the roots of the checks were clean and healthy (Fig. 2, A and B). In all cases the symptoms of the plants whose roots were infected with nematodes were different from those of plants with bunchy-top.

Further inoculations under controlled conditions were carried on by the junior author in 1927. Inoculations were made (a) by planting abacá seedlings grown from seeds in soils very heavily infected with *Heterodera radicicola* taken from bunchy-top-infected areas in Cavite; (b) by pouring around the roots of the seedlings, which were growing in sterilized soil, a suspension of from 25 to 100 nematode larvae obtained by allowing the eggs to hatch in sterile Pfeffer agar (2); and (c) by mixing with sterilized

garden soil, before abacá seedlings were planted, a quantity of chopped abacá roots which had galls in them.

The results of all of the experiments made in 1927 were exactly the same as those obtained by the senior author from 1925 to 1926. In all cases the symptoms produced on the abacá were those of root-knot, a disease which is entirely different from bunchy-top.

CONCLUSIONS

From the results of the inoculation experiments the writers conclude that the nematode *Heterodera radicicola* produces root-knot on the abacá. This disease occurs on a wide range of hosts in the tropics as well as in temperate countries. The disease is localized in the roots. Although the symptoms of root-knot are also manifest on the aërial parts of abacá, nevertheless these are very different from those of bunchy-top, which is a systemic disease of the type of the mosaics and related chloroses. There is therefore no evidence justifying the connecting of *Heterodera radicicola* with bunchy-top as heretofore reported by Hernandez (5), Teodoro (10), and Teodoro and Serrano (11 and 12).

SUMMARY

Root-knot is a very common disease of abacá, or Manila hemp, in the Philippine Islands. The disease is not usually serious. On account of the wide distribution of root-knot in abacá districts where bunchy-top occurs, the two diseases were regarded as synonymous.

Root-knot, due to *Heterodera radicicola*, causes dwarfing of abacá plants, yellowing of the foliage, reduction in size of the leaves, and formation of galls in the roots. The roots finally die and rot. The disease is entirely different from bunchy-top, showing that the nematode cannot cause this disease as heretofore reported.

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DIFFERENTIAL STAINING OF PERONOSPORACEAE[1]

E. Lepik

In the cytological work of plant pathology the differential staining of the parasites in the interior of the host is an important operation. The mycelium must form a distinct contrast to the surrounding host tissue, so that both of them can be clearly differential from each other and the pathogenic agent easily demonstrated. The success, however, is not always the same; the usual staining processes in cytology cannot be applied in every case.

In those cases in which the mycelium is distributed in the woody parts, *e.g.*, *Melampsorella caryophyllacearum* in *Abies pectinata*, the differential staining can easily be obtained with a chloride-zinc-iodine reaction. In the case of *Cronartium ribicola* in white-pine Colley (1) uses 1 per cent safranin and 1 per cent lichtgruen dissolved in 95 per cent alcohol. With Ascomycetes Vaughan (7) successfully arrived at the usual Pianeze (6) method in the cytology of higher plants.

With Peronosporaceae various staining methods have been frequently checked, whereby differential staining, too, was successful in some special cases. Mangin (4) uses benzidine dyes: benzol blue with benzolazurine and rosazurine in the alkaline bath, or Orselline BB with aniline blue in the acid bath. In the former case the hyphae are red and the host cells blue; in the latter case the reverse is true. Mangin's formula was subsequently tested by Wartenweiler (8), but he was unable to obtain differential staining on account of the poor quality of the dyes at his disposal. Otherwise he uses for the mycelium of *Plasmopara nivea* a mixture of Bavarian blue and orange, or Flemming's threefold staining. Differential stainings are not obtained thereby, only distinct pictures.

Klebahn and Philipp (5) were able to obtain good pictures in the case of *Peronospora pulveracea* Fuck. with congored, by first freeing the object from the protoplasm with Eau de Javelle. This process, however, cannot well be applied to microtome cuttings, because our present adhesives do not convey the Eau de Javelle, and segregate the cuttings from the glass.

According to Kobel (3) Uredineae and Peronosporeae can be stained with a solution of 0.1 gr. of aniline blue, 50 cc. of concentrated lactic acid and 100 cc. of water. The cuttings are left in this for five minutes, then rinsed in water, and warmed in a drop of lactic acid on the glass. The

[1] This work has been carried out with the support of the International Education Board, under the direction of Professor Dr. E. Gäumann.

mycelium takes up the stain intensely, while the host remains almost colorless. This simple process is favorable both for fresh and for herbarium material and can be applied at the same time to hand and microtome cuttings. It cannot, however, be used for permanent preparations.

When staining Peronosporaceae "bleu coton" is often used; according to Kobel (3) and Jacques Pottier this process probably originates with Prillieux. J. Pottier calls a mixture of "bleu coton GHB" and lactic acid "bleu lactique."

Klebahn[2] first stains the cuttings with "bleu coton GBBBB" in lactophenol (1 part water to 1 part glycerine, to 1 part phenol, to 1 part lactic acid) and afterwards with Orange G in oil of cloves. With this solution he obtains fine contrasts of blue and yellow.

Finally, good staining may likewise be effected in the case of Peronosporaceae with Haidenhain's haematoxyline. In objects that have been carefully differentiated with ferric alum the mycelium is bluish and the surrounding tissues are blackish. In those mycelia in which closer study of the nuclear conditions is intended, the haematoxyline method is even to be preferred to all others.

STAINING WITH BLEU COTON AND SAFRANIN[3]

Cuttings which have been obtained in the usual way by the microtome and attached to the glass by albuminous glycerine are freed from paraffin in oil of turpentine or xylol. The latter is dissipated with absolute alcohol and rinsed for a short time with 96 per cent alcohol. The glass with the cuttings is then placed in a flask with lactophenol-alcohol (I). The lactophenol[4] is composed as follows:

(I)	Phenol (carbolic acid) free of water	10 gr.
	Lactic acid, concentrated	10 cc.
	Glycerine, thickened	20 cc.
	Alcohol, 96 per cent	20 cc.

After 15 minutes the cuttings are taken from the lactophenol-alcohol and placed in the following mixture:

[2] For the particular information about his method of staining I am indebted to Professor H. Klebahn, Hamburg.

[3] The stains were supplied by the firm of Dr. Bender and Dr. Hohbein, Zurich and München, under the name of "Bleu coton 4 B," which is said to be identical with "Oxaminblau 4 BX" and "Dianylblau G."

[4] According to Amann (Ztschr. Wissensch. Mikroskopie, Bd. 13: 18. 1896), lactophenol is composed of: Carbolic acid, chem. pure, crystalline, 20 gr.; lactic acid (spec. grav. 1.31) 20 gr.; glycerine (spec. grav. 1.25) 40 gr.; distilled water 20 ccm.

(II) Bleu coton 4 B ..... 0.02 gr.
Safranin ..... 0.1 gr.
Lactophenol-alcohol (I) ..... 100 cc.

In the stain (II) the objects are left for two hours, and during this time the glass must occasionally be moved about so as to ensure equal penetration of the dye. After the cuttings have been removed from the stain (II) the lactophenol is washed away with 96 per cent alcohol, and then controlled under the microscope. If the blue staining of the mycelium is not strong enough, the cuttings must be left for a longer time in the solution. If they are too strongly stained the superfluous stain can be removed with lactophenol-alcohol. With this treatment of the cuttings red stains fade more rapidly; hence, when the proper tint of blue has been obtained, the cuttings must be left for about 10–15 minutes in a 0.5 per cent safranin solution in 96 per cent alcohol (III). They are again controlled under the microscope and, after a short rinsing with absolute alcohol, placed in xylol and then in balsam.

Better results are obtained for subsequent staining if, instead of safranin alcohol solution (III), safranin in oil of cloves is used. This process is likewise much simpler and surer. The cuttings are somewhat over-stained in solution (II), the lactophenol is washed out with absolute alcohol, and the cuttings placed in a weak safranin solution in oil of cloves. The cuttings remain in the oil of cloves until the host tissue is stained a deep red (about 20–30 min.). If over-stained, a few drops of pure oil of cloves can be used to neutralize. From the oil of cloves the cuttings are conveyed to xylol and then to balsam.

With proper treatment and differentiation—which are learned after a short experience—the host cells and the mycelium form sharp contrasts of red and blue. The qualities of both stains are easily and surely conveyed to the objects, the bleu coton fairly slowly and the safranin rapidly. Hence a commencement with safranin differentiation must only be made after that with bleu coton has been accomplished. The lactophenol-alcohol allows the cuttings to be conveyed directly from the absolute alcohol to the stain solution, and *vice versa*. Permanent preparations should be over-stained, especially with safranin, as the balsam draws off the stain.

The above process is simple in the case of serial cuttings, and the stain is durable for permanent cuttings. As a rule it is most successful with well-fixed objects (*e.g.*, Juel or Flemming fixatives).

SUMMARY

A method of differential staining is treated which has been worked out in cytological investigations of mycelium of *Plasmopara viticola* in the leaf tissue of *Vitis vinifera*, and can be well and simply applied to other Peronosporaceae.

The microtome cuttings, treated in the usual way with xylol or oil of turpentine to free them from paraffin, are dealt with as follows:

1.—10–15 minutes in lactophenol-alcohol (I);
2.—Two hours in the stain solution (II);
3.—Differentiation under the microscope, the over-stain being eliminated by lactophenol-alcohol;
4.—Wash in absolute alcohol;
5.—20–30 minutes in a weak safranin solution in oil of cloves;
6.—Differentiation under the microscope in pure oil of cloves;
7.—Wash with xylol; embed in balsam.

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PYTHIUM ARRHENOMANES N. SP., A PARASITE CAUSING MAIZE ROOT ROT

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Maize rootlets collected in the vicinity of the District of Columbia at various times during several years have yielded an assortment of nearly a dozen different species of *Pythium*. While a number of the isolations represent forms of recognized pathogenicity to a wide variety of cultivated plants, their occurrence in connection with local yellowish or brownish discolorations could not, in the absence of evidence of more serious damage, be regarded as of any considerable pathological moment. More importance presumably attaches to a form submitted for identification by Helen Johann which, in an abstract by Johann, Holbert, and Dickson,[1] was set forth as being associated with root rots of dent corn in Wisconsin and Illinois.

Although the fungus in question, as the abstract states, would seem to resemble the form to which Carpenter[2] earlier attributed root rot of sugar cane in Hawaii, the resemblance is by no means complete. Against the similarities evident between the two species with respect to the lobulate structure of the zoösporangium, the approximate dimensions of the smooth oögonium and oöspore, and, judging from Carpenter's figures, even the crook-necked shape of the terminally borne antheridium, are opposed rather significant differences in the mycelial relationships of the antheridia and in the number of these bodies to each oögonium. In the account of the cane-root parasite reference is made to "several" antheridia surrounding the female organ, while the illustrations appear to represent from 1 to 5 or at most 6 such structures. In addition it is reported that "Antheridia are often from the same branch as the oögonium." Whereas, in the maize parasite from 15 to 20 antheridia have not infrequently been counted in relation to the upper and equatorial aspects of the oögonium, indicating a probability that the total number, including those concealed underneath, might well lie between 25 and 30. Furthermore, an androgynous condition has never been observed, the mycelial connection between male and female organs being evidently relatively remote as a rule.

[1] JOHANN, H., J. R. HOLBERT and J. G. DICKSON. A Pythium seedling blight and root rot of dent corn (Abst.). Phytopath. **16**: 85. 1926.

[2] CARPENTER, C. W. Morphological studies of the Pythium-like fungi associated with root rot in Hawaii. Bul. Exp. Sta. Hawaiian Sugar Planters Assoc., Bot. Ser. 3: 59–65. 1921.

From *Pythium aphanidermatum* (Eds.) Fitz. the maize parasite differs so markedly that the possibility of specific identity is clearly out of question. In the former the almost invariably single antheridium is represented typically by a terminal, subterminal, or intercalary portion of hypha, delimited by 1 or 2 septa, together with a relatively massive orbicular, barrel-shaped or dome-shaped protuberant part making broad apical contact with the oögonium. In the latter the male organ is typically a crook-necked, expanded terminal or lateral structure making narrow contact with the oögonium and never set off from its supporting filament by more than a single cross-wall. It may be noted that inasmuch as in Carpenter's plates the second type of antheridium is illustrated, the conclusion seems unescapable that the identification of the cane parasite as *Pythium aphanidermatum* was quite erroneous.

While the maize root fungus under consideration apparently is not recognizable in any published descriptions based on material from the underground parts of sugar cane, it is not intended to assert at this time that the pathogen may not also occur there. A goodly proportion of several hundred cultures from affected sugar cane roots, obtained in part from greenhouse material collected at Arlington Experiment Farm, Rosslyn, Virginia, but mostly isolated in Louisiana by R. D. Rands from field material, exhibit general similarities to the maize parasite in mycelium and zoösporangial complexes. This is true also of many of the isolations from corn roots to which reference has been made. Final opinion concerning the probable relationship of such forms from the two hosts must await the development of the sexual stage, and that on a substratum permitting satisfactory microscopic study.

Isolations from diseased maize rootlets submitted by W. D. Valleau as typical of the root rot described from Kentucky by Valleau, Karraker, and Johnson[3] yielded a preponderance of the "Pythium-like fungus" represented in their illustrations. Its similarity in both sexual and asexual phases is strongly indicative of specific identity with the Wisconsin parasite, which latter may expediently serve as type of a new species for which the term *arrhenomanes*, suggestive of the extraordinary supply of male elements, is proposed.

Pythium arrhenomanes n. sp.

Mycelium intercellular and intracellular; in culture exhibiting moderately strong aerial development; composed of hyphae 2.0–5.5 μ in diameter. Zoösporangia lobulate, composed of inflated communicating elements up to

[3] VALLEAU, W. D., P. E. KARRAKER and E. M. JOHNSON. Corn root rot—a soil-borne disease. Jour. Agr. Res. 33: 453–476. 1926.

20 μ or more in diameter, often occurring in extensive compound complexes; evacuation tube usually 3 to 4 μ in diameter, variable in length (frequently 50–75 μ); zoöspores usually from 20 to 50 or more from a vesicle, 2-ciliated, motile, later rounding up as subspherical or ellipsoidal bodies with average diameter of approximately 12 μ, and germinating usually by a single germ tube 2.5 to 3.0 μ in diameter.

Oögonia (on carrot-cornmeal agar) subspherical, terminal or more rarely intercalary, measuring 24–35 μ (average 29.4 μ) in diameter, with a wall approximately 0.5 μ in thickness. Antheridia crook-necked, measuring usually 6–9 μ in diameter in the distal expanded portion, 12–25 μ in length along curved axis from apex to basal septum, the rounded apical end making narrow contact with oogonium about a short fertilization tube that measures approximately 3 μ in diameter, the proximal part more gradually tapering toward delimiting septum to diameter of supporting filament; numerous, from 15 to 20 often visible in relation to an oögonium, the total number then probably in excess of 25; borne terminally or more rarely laterally on branches arising from several (usually 4–8) hyphae, each of which contributes usually up to 4 antheridia, and all of which are distinct from hypha bearing oögonium. Oöspores (on carrot-cornmeal agar) subspherical, yellowish, sometimes completely filling oögonium, usually 22–33 μ (average 27.3 μ) in diameter, containing a reserve globule often 12–19 μ (average 15.4 μ) in diameter, and surrounded by a wall 1.2–2.0 μ (average 1.6 μ) in thickness.

Causing a decay of maize (*Zea mays* L.) roots in Wisconsin.

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